U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-268 538

Extension of Multi-Residue Methodology. I Determining Multiclass Pesticide Residues in Soil by Gas Chromatography. II. Dynamic Fluorogenic Labelling Detector for Carbamates

Florida Univ., Gainesville

Prepared for

Health Effects Research Lab., Research Triangle Park, N.C.

May 77

EPA-600/1-77-028
May 1977

PB 268 538

Environmental Health Effects Research Series

EXTENSION OF MULTI-RESIDUE METHODOLOGY:

[illegible]
NATIONAL TECHNICAL
INFORMATION SERVICE
[illegible]

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ENVIRONMENTAL HEALTH EFFECTS RESEARCH series. This series describes projects and studies relating to the tolerances of man for unhealthful substances or conditions. This work is generally assessed from a medical viewpoint, including physiological or psychological studies. In addition to toxicology and other medical specialities, study areas include biomedical instrumentation and health research techniques utilizing animals — but always with intended application to human health measures.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-600/1-77-029

2.

3. RECIPIENT'S ACCESSION NO.

4. TITLE AND SUBTITLE
EXTENSION OF MULTI-RESIDUE METHODOLOGY. I. Determining Multiclass Pesticide Residues in Soil by Gas Chromatography. II. Dynamic Fluorogenic Labelling Detector for Carbamates.

5. REPORT DATE
May 1977

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
H. Anson Moye, Sujit Witkonton and Gordon Cash

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
University of Florida, IFAS
Food Science Department
Pesticide Research Laboratory
Gainesville, FL 32611

10. PROGRAM ELEMENT NO.
1EA615

11. CONTRACT/GRANT NO.
68-02-1706

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory RTP,NC
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

A multi-residue procedure was devised for the extraction, cleanup and determination of forty seven pesticides in fortified soil samples. Most of the compounds were determined by gas chromatography interfaced with eithur the electron capture or the flame photometric detector. Several nitrogen containing pesticides were quantitated using a fluorescence detector in conjunction with a high performance liquid chromatographic system. Various methods of extraction were compared for several pesticides. A silica gel column, eluted sequentially with various solvents, was used for cleanup and separation of compounds into groups. Several different derivatization procedures were used to render some pesticides more amenable to gas chromatographic detection. A dynamic fluorogenic labelling detector was designed and characterized for the high pressure liquid chromatographic analysis of six N-methylcarbamate and two carbamoyl oxime pesticides. Lannate, Matacil, Temik, Baygon, carbofuran, Sevin and Mesurol could be extracted from sandy soil at the 0.01 ppm level with recoveries ranging from 83 to 115%. Somewhat lower recoveries were experienced from sandy loam and silty loam soils. Zectran could not be reproducibly chromatographed under the liquid chromatographic conditions chosen for the separations. No cleanup was required prior to the liquid chromatography of the soil extracts; no significant interferences were observed for the unclean extracts.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pesticides extraction chemical analysis gas chromatography soil analysis		14 B 07 A

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES [illegible]
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE A09/MF A01

EPA Form 2220-1 (9-73)

EPA-600/1-77-029
May 1977

EXTENSION OF MULTI-RESIDUE METHODOLOGY
I. DETERMINING MULTICLASS PESTICIDE RESIDUES IN SOIL BY GAS CHROMATOGRAPHY
II. DYNAMIC FLUOROGENIC LABELLING DETECTOR FOR CARBAMATES

by

H. Anson Moye, Sujit Witkonton and Gordon Cash
University of Florida, IFAS. Food Science Department
Pesticide Research Laboratory, Gainesville, FL 32611

Contract No.

68-02-1706

Project Officer

Robert F. Moseman
Environmental Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711

1-a

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This report represents a research effort to extend and improve analytical methodology for the determination of a variety of pesticide residues in our environment. The emphasis is on less persistent chemicals which are being used in place of the more environmentally stable pesticides.

John H. Knelson, M.D.
Director,
Health Effects Research Laboratory

ABSTRACT

Of all the forty pesticides under investigation, twelve (methyl parathion, parathion, malathion, phorate, azinphos methyl, azinphos ethyl, Dursban^{R}, diazinon, dimethoate, phosphamidon, Azodrin^{R} and demeton) were detected by the FPD-P detector, two (Temik^{R} and methomyl) by the FPD-S detector, fourteen (captan, chlorobenzilate, endosulfan, Avadex^{R}, folpet, methoxychlor, PCNB, Perthane^{R}, trifluralin, atrazine, bromacil, CIPC, Difolatan^{R} and simazine) by EC detector. Twelve have to be derivatized by PFPA (bux, carbofuran, monuron, Zectran^{R}, Landrine^{R} and IPC), PFBC (benomyl, monuron and amitrole) or DAM (DNOC, DNBP, and dicamba) and then detected by the EC detector. The 4% SE-30/6% OV-210 column exhibited superior separation efficiency for most pesticides than other tested columns. The usefulness of the nitrogen-specific Hall detector was limited by its erratic performance. It was caused by the repetitive injection of unvented solvent and subsequent acid contamination in the detection system.

The silica gel column was proven to be consistent and reliable for soil sample cleanup and separation of multi-class pesticides. Some impurities from blank silica gel columns were present in various eluted fractions; however, this drawback was offset by their effectiveness in cleanup and separation of various pesticides from soil co-extractants.

Good recovery results were obtained by the Soxhlet procedure for most of the organophosphate and halogenated pesticides,

except for demeton, bromacil, endosulfan and trifluralin. Also, captan, folpet and Difolatan reacted with methanol during Soxhlet extraction; however, they were subsequently extracted and recovered by ambient tumbling or Polytron^{R} ultrasonic extraction.

Most of the heat-labile N-containing pesticides were extracted by the Polytron and recovered in good yield except amitrole, Temik and methomyl. Figure 29 summarized the procedure for detection of forty multi-class pesticides in soils.

A dynamic fluorogenic labelling detector was designed and characterized for the high pressure liquid chromatographic analysis of six N-methylcarbamate and two carbamoyl oxime pesticides. Lannate, Matacil, Temik, Baygon, carbofuran, Sevin and Mesurol could be extracted from sandy soil at the 0.01 ppm level with recoveries ranging from 83 to 115%. Somewhat lower recoveries were experienced for sandy loam and silty loam soils. Zectran could not be reproducibly chromatographed under the liquid chromatographic conditions chosen for the separations. No cleanup was required prior to the liquid chromatography of the soil extracts; no significant interferences were observed for the unclean extracts.

This report was submitted in fulfillment of Contract No. 68-03-1706 by Pesticide Research Laboratory, Food Science Department, IFAS, University of Florida under the sponsorship of the U.S. Environment Protection Agency. This report covers the period June 14, 1974, to June 26, 1976, and work was completed as of June 26, 1976.

CONTENTS

Foreword iii

Abstract iv

Figures viii

Tables xiii

Extension of Multi-residue Methodology. I. Determining Multiclass Pesticide Residues in Soil

Introduction 1

Experimental 9
 Apparatus 9
 Reagents. 11
 Pesticide Standard Solutions. 13
 Pesticide Derivatization. 13
 Preparation of Spiked Soil Samples and Extraction Procedure 16
 Polytron Ultrasonic Extraction Procedure. 16
 Silica Gel Column Chromatography. 17

Results and Discussion 18
 Screening of Pesticides for GLC responses 18
 Derivatization of Pesticides. 48
 GLC Detection of S-containing Pesticides. 70
 Loss of Pesticides in the Concentration Steps . . . 71
 Silica gel Column Chromatography. 74
 Co-extractive Interferences from Crude Soil Extracts and Cleanup by Silica Gel Column Chromatography. 79
 Recoveries of Pesticides from Soils by Soxhlet Extraction 89
 Recoveries of Some Organophosphate and Thermally Labile N-containing Pesticides from soil by Polytron Ultrasonic Extraction and Comparison of Results with Soxhlet Extraction 103
 Stimultaneous Analysis of Forty Multi-class Pesticides in Sandy Soil 119
 References. 130

Extension of Multi-residue Methodology. II. Dynamic Fluorogenic Labelling Detector for Carbamates

Introduction 132

Experimental 134
    Apparatus and Reagents 134
    HPLC Column Study 136
    Studies of Fluram 140
    Studies on o-Phthalaldehyde 142

Conclusions. 162

References 167

FIGURES

Extension of Multi-residue Methodology. I.

Number		Page
1a	Chromatograms of 14 EC-sensitive pesticides on 4% SE 30/6% OV-210 column	24
1b	Chromatograms of 14 EC-sensitive pesticides on 1.5% OV-17/1.95% OV-210 column	25
1c	Chromatograms of 14 EC-sensitive pesticides on 5% OV-210 column	26
2	Analytical curve of Avadex	27
3	Analytical curve of captan, folpet and Difolatan	28
4	Analytical curves of Perthane, chlorobenzilate and CIPC	29
5	Analytical curves of trifluralin and bromacil	30
6	Analytical curves of simazine and atrazine	31
7	Analytical curves of PCNB, endosulfan and methoxychlor	32
8	Analytical curves of Azodrin	41
9	Analytical curves of Dursban, dimethoate and phosphamidon	42
10	Analytical curve of diazinon	43
11	Analytical curves of phorate and dimeton	44
12	Analytical curves of malathion and parathion	45
13	Analytical curves of azinphos ethyl, azinphos methyl and methyl parathion	46
14	Analytical curve of PFPA-IPC	54
15	Analytical curves of carbofuran, bux, Zectran, Landrins (PFPA)	55
16	Analytical curve of PFBC-amitrole	63

FIGURES

Number Page

17 Analytical curve of PFBC-benomyl 64

18 Analytical curve of PFBC-monuron 65

19 Analytical curves of dicamba, DNOC and DNBP (DAM) 66

20 Analytical curves of Temik and methomyl (4% SE 30/5% OV-210) 72

21 Analytical curves of Temik and methomyl (5% Carbowax 20 M) 73

22 Chromatogram of standard PFPA-derivatives of six carbamate pesticides (a), and after they were heated at 100°C in a sealed test tube for 6 hr in 1:1 benzene/MeOH (b) 78

23a Outlines of halogenated and organophosphate pesticide standards (1 mg each) eluted from silica gel column 80

23b Outline of multi-class pesticide standard (1 mg) eluted from silica gel column 81

24a Gas chromatograms of crude control sandy soil extract (10 mg soil equivalent) 83

24b Gas chromatograms of control and 0.01 ppm PCNB from sandy soil (10 mg soil equivalent) 84

24c Gas chromatograms of 0.01 ppm in sandy soil of Avadex (3), trifluralin (39), endosulfan (21), methoxychlor (29), and Perthane (35) in 60% benzene/methanol fraction and the respective control (50 mg soil equivalent) 85

24d Gas chromatograms of 0.01 ppm in sandy soil of CIPC (12), captan (10), folpet (22), chlorobenzilate (11) and Difolatan (16) and the respective control (10 mg soil equivalent) 86

24e Gas chromatograms of 0.01 ppm in sandy soil of atrazine (2) and simazine (37) and the respective control (50 mg soil equivalent) 87

FIGURES

Number		Page
24f	Gas chromatograms of 0.01 ppm in sandy soil of bromacil (8) and the respective control (50 mg soil equivilent)	88
25a	Gas chromatograms. Top; control (500 mg soil equivalent). Middle; 0.01 ppm OP pesticides in fraction II (60% B/H). Bottom; fraction III (5% A/B)	90
25b	Chromatograms of 0.01 ppm OP pesticids in fraction IV (10% A/B), fraction V (20% A/B) and fraction VI (100% A/B)	91
25c	Gas chromatograms of 6 FPD (P) sensitive pesticides from 4% SE 30/6% OV-210 column	92
25d	Gas chromatograms of 6 FPD (P) sensitive pesticides from 4% SE 30/6% OV-210 column	93
26	Chromatograms of control crude sandy soil (2 mg equivalent) and recoveries of 0.01 ppm of dicamba (13), DNOC (18) and DNBP (19) by diazomethane derivatization	95
27a	Chromatograms, left; of 1 ppm Zectran (40) and the respective control, right; 1 ppm IPC (24) and its control (1 mg equivalent), PFPA derivatives	96
27b	Chromatograms of 0.1 ppm bux (9) and its respective control (1 mg soil equivalent), PFPA derivative	97
27c	Gas chromatograms, of Landrins (25,26) and the respective control (1 mg soil equivalent) PFPA derivatives	98
27d	Chromatograms of 0.1 ppm carbofuran and its control	99
28a	Chromstograms of PFBC-control (5 mg soil equivalent)(83%) of 0.01 ppm monuron from sandy soil	100
28b	Chromatograms of PFBC-control (10 mg soil equivalent) and recovery of 0.1 ppm benomyl from sandy soil	101
29	Procedure for detection of forty pesticides in soil	129

Extension of Multi-residue Methodology. II.

Figure		Page
1	Isocratic separation of six carbamate mix on μC_{18}, 40% acetonitrile - 60% H_2O	138
2	Isocratic separation of early eluting carbamates on μCN, 12.5% acetonitrile - 87.5% H_2O	139
3	Isocratic separation of late eluting carbamates on ODS, 12.5% acetonitrile - 87.5% H_2O	139
4	Relative fluorescence of N-methylcarbamates as a function of time	144
5	Relative fluorescence of N-methylcarbamates as a function of time	144
6	Relative fluorescence of N-methylcarbamates as a function of time	145
7	Relative fluorescence of N-methylcarbamates as a function of time	145
8	Modular dynamic fluorogenic labelling liquid chromatograph	147
9	Chromatograms, Top; of sandy soil, spiked at 0.01 ppm. Program No. 10 for 15 min, 25% dioxane - H_2O to 40% dioxane - H_2O, 1 ml/min. Bottom; check sandy soil	149
10	Chromatogram of sandy loam soil	149
11	Chromatogram of silty loam soil	150
12	Chromatogram of sandy soil spiked at 1.0 ppm . . .	150
13	Chromatogram of sandy loam soil	151
14	Chromatogram of silty loam soil	151
15	Analytical curves for six N-methylcarbamate pesticides	154
16	Effect of withholding OPA (substituting pH 10 buffer) on response of celery spiked at 0.2 ppm with 1, Lannate; 2, Temik; 3, Baygon; 4, carbofuran; and 5, Sevin	156

Figure Page

17 Effect of withholding NaOH (substituting pH 7 H_2O) on response of lettuce spiked at 0.2 ppm with: 1, Lannate; 2, Temik; 3, Baygon; 4, carbofuran; 5, Sevin; and 6, Mesurol 156

18 Plot of 4 σ (peak width at base) for contributions of hydrolysis coil and of fluorometer cell, as a function of flow through each 157

19 Aminco Aminalyzer, adapted to N-methylcarbamate analyses 163

20 Aminalyzer chromatogram of: 1, Lannate; 2, Temik; 3, Baygon; 4, carbofuran; 5, Sevin; and 6, Mesurol 164

21 Aminalyzer extended range analytical curve of Lannate 164

TABLES

Extension of Multi-residue Methodology. I.

Number | Page

1 List of pesticides under multi-residue methodology investigation 2

2 Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 10% DC-200. 19

3 Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 10% QF-1 20

4 Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 4% SE 30/6% OV-210 21

5 Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 1.5% OV-17/1.95% OV-210 22

6 Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 5% OV-210 23

7 Computed efficiencies of five GLC columns 36

8 Retention times and response values relative to parathion of eight OP pesticides. 37

9 Retention times and response values relative to parathion of twelve organophosphate pesticides on 4% SE-30/6% OV-210, 200°C 38

10 Retention times and response values relative to parathion of twelve organophosphate pesticides on 1.5% OV-17/1.95% OV-210, 200°C 39

11 Retention times and response values relative to parathion of twelve organophosphate pesticides on 5% OV-210, 200°C 40

12 Retention times and response values relative to atrazine, of Nitrogen-containing pesticides on 5% SE-30 column, 180°C (Hall detector) 49

TABLES

Number **Page**

13 Retention times and response values relative to aldrin of PFPA derivatized pesticides on two columns (Packard 7820, EC) 50

14 Retention times and response values relative to aldrin of PFPA derivatized pesticides on two columns (Tracor 222, EC) 51

15 Retention times and response values relative to aldrin of 2,5-dichlorobenzenesulfonyl chloride (pyridine system) derivatized pesticides on two GLC columns 52

16 Retention times and response values relative to aldrin of PFBC derivatized pesticides (pyridine system) on 10% DC-200 column, 205°C 57

17 Retention times and response values relative to aldrin of PFBC derivatized pesticides (pyridine system) on 4% SE-30/6% OV-210 column 58

18 Retention times and response values relative to aldrin of PFBC derivatized pesticides (pyridine system) on 1.5% OV-17/1.95% OV-210 column 59

19 Retention times and response values relative to aldrin of PFBC derivatized pesticides (0.1 M NaOH system) on 1.5% OV-17/1.95% OV-210 60

20 Retention times and response values relative to aldrin of PFBC derivatized pesticides (0.1 M NaOH system) on 4% SE-30/6% OV-210 61

21 Retention times and response values relative to aldrin of BPFT derivatized pesticides on two columns 67

22 Retention times and response values relative to aldrin of diazomethane derivatized pesticides on 4% SE-30/6% OV-210 68

23 Retention times and response values relative to aldrin of selected derivatization techniques on twelve multi-class pesticides 69

24 Percent recovery of 500 µg of pesticides each in the Kuderna-Danish concentrator step concentrated from 250 ml 1:1 benzene/methanol to 10 ml 75

TABLES

Number **Page**

25 Effect of paraffin oil keeper on pesticide retention 76

26 Recovery of 100 µg of nitrogen containing pesticides after heating for 6 hr (100°C) in a capped test tube with 2 ml benzene/methanol (1:1) . 77

27 Average percent recoveries of phorate and parathion from sandy soil which were spiked at 0.00, 0.01, 0.10, 1.00 and 10.00 ppm 102

28 Soil characterization 104

29 Average percent recoveries of halogenated pesticides from silty loam soil which were spiked at 0.01, 0.10, 1.00 and 10.00 ppm 105

30 Average percent recoveries of halogenated pesticides from sandy soil which were spiked at 0.01, 0.10, 1.00 and 10.00 ppm 106

31 Average percent recoveries of halogenated pesticides from sandy loam soil which were spiked at 0.01, 0.10, 1.00 and 10.00 ppm 107

32 Average percent recoveries of organophosphate pesticides from sandy soil which were spiked at 0.01, 0.10, 1.00 and 10.00 ppm 109

33 Average percent recoveries of organophosphate pesticides from sandy loam soil which were spiked at 0.01, 0.10, 1.00 and 10.00 ppm 110

34 Average percent recoveries of organophosphate pesticides from silty loam soil which were spiked at 0.01, 0.10, 1.00 and 10.00 ppm 111

35 Comparison of solvents for ultrasonic-Polytron extraction of carbofuran from sandy soil 112

36 Effect of Polytron extraction time on recovery of carbofuran from sandy soil 114

37 Effect of Polytron motor speed on recovery of carbofuran from sandy soil. 115

TABLES

Number		Page
38	Average percent recoveries of pesticides from sandy soil samples which were spiked at 0.01 and 0.10 ppm (Polytron extraction)	116
39	Average percent recoveries of pesticides from sandy soil samples which were spiked at 0.01 and 0.10 ppm (Soxhlet extraction)	117
40	Average percent recoveries of pesticides from sandy soil samples which were spiked at 0.01 and 0.10 ppm (Polytron extraction)	120
41	Average percent recoveries of pesticides from sandy soil samples which were spiked at 0.01 and 0.10 ppm (Soxhlet extraction)	121
42	Multi-residue analysis of fourteen EC-sensitive pesticides in duplicate sandy soil samples which were spiked at 0.01 and 0.10 ppm of forty multi-class pesticides	122
43	Multi-residue analysis of twelve FPD-P sensitive pesticides in duplicate sandy soil samples which were spiked at 0.01, and 0.10 ppm of forty multi-class pesticides	125
44	Multi-residue analysis of twelve multi-class/nitrogen containing pesticides in duplicate sandy soil samples which were spiked at 0.01 and 0.10 ppm	126

Extension of Multi-residue Methodology. II.

Number		Page
1	Lower limits of detection for seven carbamates in static Flram system	141
2	Soil recoveries for seven carbamates for silty loam, sandy loam and sandy soils	159

Extension of Multi-residue Methodology. I. Determining Multiclass Pesticide Residues in Soils by Gas Chromatography

H. Anson Moye and Sujit Witkonton

Introduction

The exceptionally large number of pesticides, representing a diversity of compound classes, presents a severe problem to those charged with their analyses. Efforts to extend the existing multi-residue methodology, largely compiled by EPA and FDA, to these compounds and substrates presently not included would certainly be in order for a number of reasons, which will not be elaborated here.

Multi-residue methodology, due to its inception by the FDA, historically has concerned itself with the analysis of foods, primarily utilizing a gas chromatographic determinative step. Introduction of newer pesticides, along with a growing need to know of their presence in non-target systems (the environment) has limited the body of multi-residue methodology in terms of its applicability, partly because these compounds have not been thoroughly tested through the multi-residue methods and partly because those that have been tested have been difficult to detect gas chromatographically. A large number, if not all, of those compounds listed in Table I would certainly fall into one or both of these categories.

Table 1. List of Pesticides* under Multi-residue Methodology Investigation

Number	Pesticide	Number	Pesticide	Number	Pesticide
1	Amitrole	14	Diazinon	27	Malathion
2	Atrazine	15	Dicamba	28	Methomyl
3	Avadex^R (diallate)	16	Difolatan^R (captafol)	29	Methoxychlor
4	Azinphos ethyl	17	Dimethoate	30	Methyl parathion
5	Azinphos methyl	18	DNOC	31	Monuron
6	Azodrin^R (monocrotophos)	19	DNBP	32	Parathion
7	Benomyl	20	Dursban^R (chlorpyrifos)	33	Phosphamidon
8	Bromacil	21	Endosulfan	34	PCNB
9	Bux^R (metalkamate)	22	Folpet	35	Perthane^R (ethylan)
10	Captan	23	Furadan^R (carbofuran)	36	Phorate
11	Chlorobenzilate	24	IPC^R (propham)	37	Simazine
12	CIPC^R (chlorpropham)	25	2,3,5-Landrin^R (trimethylphenyl methylcarbamate)	38	Temik^R (aldicarb)
13	Demeton	26	3,4,5-Landrin^R	39	Trifluralin
				40	Zectran^R (mexacarbate)

*Where trade names are used, the common name appears in parentheses. Certain trade names are probably more familiar to many readers than the designated common names. For this reason, some pesticides will be referred to by their trade names through out the text.

Although techniques other than gas chromatography have been used in the determinative steps of pesticide methodology schemes, they usually lack sensitivity and selectivity. Usually the inability to perform a gas chromatographic analysis on an organic pesticide can be attributed to thermal instability or lack of volatility. These problems are well known for the carbamates, carbamoyl oximes, phosphonates and some substituted ureas, and prevent full utilization of the commercially available detectors, many of which would otherwise provide adequate sensitivity and selectivity. The use of chemical derivatives obviates these problems in many cases (1-5).

The multi-residue method of FDA, later used and extended by EPA to non-food samples, is largely based upon the extraction and cleanup procedure reported by Mills, Onley and Gaither in 1963 (6). This procedure could be applied to those foods of 2% fat content or less; however, subsequent modifications have covered essentially all food types (7-10). The Pesticide Analytical Manual (PAM) of the Food and Drug Administration contains a fairly comprehensive list of those pesticides which have, or have not, been tested through what has become known as the "Mills" procedure. Most of the compounds listed in Table I either were not completely recovered through the procedure or were simply not tested.

Since the main responsibilities of the FDA have not included soil, no information concerning its extraction and cleanup is found within the PAM. Extraction methods in large variety, however, have been reported in the pesticide analytical literature utilizing the full spectrum of solvents and techniques from simple swirling to lengthy Soxhlet extraction. Probably the most extensive review of the field to date is that of Chiba (11) in Residue Reviews, which presents somewhat conflicting data on soil extraction efficiencies. It would be hoped that since this project concerns itself with the extension of existing methodology, those solvents used prior to the Mills Florisil column would perform adequately as extractants of soil. Unfortunately, according to those works reviewed by Chiba, neither acetonitrile nor petroleum ether (both used in the Mills procedure) are satisfactory. Chiba found that solvent pairs, one nonpolar and ther other polar, were more satisfactory than any other arrangement for the extraction of air dried sandy loam soil. A great many factors contribute to the success with which soils may be extracted, including soil type, moisture content, agitation, contact time and solvent. These must all be considered in any efficiency study.

Success in gas chromatographing involatile or thermally unstable compounds can often be achieved with one or both of the following approaches: (1) injection of relatively large amounts of pesticide, approaching one microgram, so that chemically active sites on the column are momentarily masked, resulting in a higher percentage of the pesticide reaching the detector, or (2) chemical derivatization usually at an ionizable function group, so that volatility and thermal stability are improved. The first approach is made more feasible by the recent improvements in element specific detectors, such as the flame photometric, the electrolytic conductivity and the microcoulometric detectors. These types of detectors possess such large inherent selectivity that relatively large amounts of sample can be injected without interferences becoming prohibitive. Selectivity can also be achieved in the second approach by making derivatives which are not only characteristic of certain functional groups or molecular structures but also give responses with element selective detectors or the electron capture detector.

To meet the above objective, sequences and scope of research work were designed as follows:

1. Screening of pesticides listed in Table 1 for gas chromatographic response. The halogen containing pesticides were checked with the tritium electron capture detector (EC), the phosphorus and sulfur containing pesticides with the flame photometric detector (FPD) and the nitrogen containing pesticides with the electrolytic conductivity detector. Five columns of 10% DC-200, 10% QF-1, 4% SE-30/6% OV-210, 1.5% OV-17/1.95% OV-210, and 5% OV-210 and their operating conditions for chlorinated hydrocarbons which were specified by PAM were tried.

2. Pesticide derivatization. Pesticides which gave poor or no response to gas liquid chromatography (GLC) were initially derivatized by applying the perfluoroacylation method (12) and halogenated-benzene-sulfonates formation (13). GLC conditions that are normally used for the nonderivatized pesticides

were used.

3. Soil sample preparation. Pesticides which gave acceptable GLC response were studied for recovery in sandy, sandy loam and silty loam soils. Triplicate samples of 50-100 g of soil were spiked at 0.00, 0.01, 0.10, 1 and 10 ppm, extracted after curing for 30 minutes, then analyzed.

4. Recovery of pesticides from soil. One hundred gm of each soil sample was extracted by Soxhlet extraction with 300 ml of benzene-methanol (1:1).

5. Cleanup of soil extracts. Soil extracts were cleaned up by silica-gel column chromatography. Alternate solvent systems were examined in order to obtain maximum recovery of pesticides. Results are presented in average percent recoveries with specific accompanying chromatograms to show degree and extent of co-extractive interferences.

6. Special efforts were directed at finding suitable derivatives for those pesticides which during the past year's (1974-75) work have been unsuccessfully gas chromatographed (Table 1). Nine additional pesticides were requested by EPA to be included in the 1975-76 multi-residue investigation. For those compounds which appeared appropriate (possess appropriate functional groups), the following derivatization reagents were tested: (1) N-methyl-bis-(heptafluorobutyryl) amide, (2) 9-chloromethyl anthracene, (3) pentafluorobenzoyl chloride, (4) O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride, (5) acetic acid-N-hydroxysuccinimide ester, (6) α-bromo-2,3,4,5,6-pentafluorotoluene, (7) diazomethane, (8) pentafluoropropionic

anhydride, and (9) N-methyl-bis-(trifluoroacetamide). When reagents became available during the course of the contract period and appeared to offer advantages over those listed above, they were also tested.

Standard procedures were used to attempt derivatization on a micro scale. Aliquots of the appropriately manipulated reaction solution were injected onto three gas chromatographic columns (4% SE-30/6% OV-210, 1.5% OV-17/1.95% OV-210, and 5% OV-210), specified by EPA, installed in a Tracor 222 gas chromatograph equipped with dual tritium EC detectors. For those pesticides which gave responses, analytical curves were made and limits of detection established (three times noise). Reproducibility of derivatization and gas chromatography was also established.

Once it had been determined which problem compounds were amenable to gas chromatography by derivatization, a soil extraction study was undertaken. Emphasis was on determining the feasibility of utilizing an ultrasound extraction device (29), the Willems Polytron PT 20, and comparing its performance to that of Soxhlet extraction. Since the three soil types in the 1974-75 work behaved similarly in terms of extraction efficiencies, only the soil giving the most gas chromatographic interference was studied, i.e. the sandy soil.

Preliminary to extracting all the problem compounds, one compound was selected to use for optimizing the extraction technique for the Polytron. Once the optimum extraction technique had been established, the problem compounds were applied as standards to the silica gel columns so as to determine in which eluted fraction they appeared. Subsequently each compound was added to soil, aged for 30 min, extracted with the Polytron, derivatized, if necessary, and analyzed gas chromatographically. Percent recoveries were compared to those already obtained by Soxhlet extraction. However, for those compounds which could be derivatized with one of the proposed reagents, Soxhlet extractions were made for comparison.

Experimental

Apparatus

Electron Capture Detector. Packard model 7820 gas chromatograph equipped with two tritium foil electron capture detectors, two independent recorders and gas chromatographic oven Packard model 802, was operated under the following conditions:

Two 1.8 m x 4 mm I.D. coil columns packed with 10% DC-200 and 10% QF-1 on Gas Chrom Q, 80-100 mesh; inlet temperature 220°C; outlet, 230°C; detector, 220°C; column, 195°C.

Electron Capture Detector. Tracor 222 gas chromatograph equipped with two tritium foil electron capture detectors and two independent Soltec recorders was operated under the following conditions:

Two 1.8m x 2 mm I.D. U-shaped glass columns packed with either pair of 10% DC-200, 10% QF-1, 4% SE-30/6% OV-210, 1.5% OV-17/1.95% OV-210 or 5% OV-210 on Gas Chrom Q, 80-100 mesh; inlet temperature 230°C; detector, 220°C; column, 205°C.

Flame Photometric Detector. Varian Aerograph series 1200 equipped with Tracor flame photometric detector, Soltec recorder 1 mv, high voltage supply and electrometer of Keithley instruments, was operated under the following conditions:

1.8 m x 2 mm I.D. coil-shaped glass columns packed with the same column packings as for the EC detectors. The packing was topped with 2.5 cm of 10% Carbowax 20 M on Chromosorb W (HP), 80-100 mesh; inlet temperature, 250°C (300°C for Temik and methomyl); detector, 165°C; nitrogen carrier gas flow rate, 50 ml/min; O_2, 15 ml/min; air, 50 ml/min; H_2, 150 ml/min; high voltage setting, 850 volts. Phosphorus or sulfur mode. The FPD was modified by reversing the H_2 and air/O_2 inlets so that the flame would not be extinguished by solvent after each injection.

The sulfur mode of FPD was modified in order to increase its sensitivity by the introduction of SO_2 (25). The SO_2/N_2 (1.46 ppm of SO_2) auxiliary gas was mixed through the H_2 line by means of a T-connector located approximately 15 cm before the detector. The auxiliary gas pressure was adjusted to 50 psig. and the flow was controlled at 50 ml/min with a metering valve.

Hall Electrolytic Conductivity Detector, Model 310. Tracor 222 gas chromatograph was equipped with one Hall electrolytic conductivity detector and a Soltec recorder. The gas chromatograph was operated under the following conditions:

1.8 m x 2 mm I.D. U-shaped glass column packed with 5% SE-30 on Chromosorb W (HP), 80-100 mesh; inlet temperature, 220°C; column, 186°C; transfer line, 270°C; Helium gas flow rate, 67 ml/min. Hall detector settings: Furnace temperature, 820°C; Mode: Nitrogen; Reaction gas: Hydrogen, 35 ml/min; cell flow, 0.5 ml/min. attenuation, 1 x 10.

Chromaflex column, size 50-25, Kontes K-422250.

Kuderna-Danish evaporative concentrator, 500 ml.

Culture tube, 5 ml, Scientific Products, catalog no. T1346-5.

Microliter pipets, 10, 25, 50, 100, 200, and 500 µl. Eppendorf.

Soxhlet extraction apparatus; Kimax brand (24005), flask, 500 ml. Fisher Scientific Co. catalog no. 9-551C.

Extraction thimbles, glass. Fisher Scientific Co. catalog no. 9-655C.

Kerr wide mouth mason jars, 12 quarts.

Disposable pasteur pipets.

Unitized Extraction Assembly (Precision 65500). Fisher Scientific Co. catalog no. 9-678.

Nitrogen evaporator with hot plate maintained at 40-50°C.

Fisher Isotemp^{R} dry bath, model 145.

Automatic shaker of Eberback Corporation, Ann Arbor, Mich.

Automatic tumbling assembly.

Maxi-Mix model M-16715, series 16700, Thermolyne Sybron Corporation.

Willems Polytron, model PR-20, Brinkmann Instruments, Westburg, New York.

Reagents

Burdick & Jackson's "distilled in glass" or redistilled solvents: hexane, benzene, acetonitrile, methanol, acetone.

Anhydrous sodium sulfate, acetone-extracted.

Pyridine, redistilled.

Silica gel dry column, activity III, (20% H_2O) ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio, catalog no. 404526.

Pentafluoropropionic anhydride, PCR, Inc., Research Chemicals Division, Gainesville, FL, catalog no. 13670.

2,5-dichlorobenzenesulfonyl chloride, Eastman Kodak Co., Rochester, NY 14650. Recrystallized three times from redistilled isooctane.

N-methyl-bis-(heptafluorobutyryl) amide, N-methyl-bis-(trifluoroacetamide). Regis Chemical Co., 8210 N. Austin Ave., Morton Grove, Illinois.

Pentafluorobenzoyl chloride, 98% pure, α-bromo-2,3,4,5,6-pentafluorotoluene, 99+% pure, 9-Anthracene-methanol, Aldrich Chemical Co., 3355 Lenox Rd., N.E., Suite 750, Atlanta, Georgia.

Thionyl chloride, Mallinckrodt Chemical Works, St. Louis, Missouri.

Acetic acid N-hydroxysuccinimide ester, ICN Pharmaceuticals, Inc., Life Science group, Cleveland, Ohio.

O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine, Applied Science Laboratories, Inc., State College, Pennsylvania.

Diazomethane in ether, prepared from "Diazald," Aldrich Chemical Co., Inc. (Synthesis procedure obtained from Aldrich's bulletin).

Phosphate buffer (pH 7): 3 g NaOH and 17 g KH_2PO_4 in 200ml water.

Keeper solution (1% paraffin oil in benzene).

Pesticide Standard Solutions

Ten mg of each analytical grade pesticide (provided by EPA) was dissolved in 10 ml of redistilled benzene and kept as concentrate stock solution (1 mg/ml). For those compounds which could not be dissolved readily, benzene/acetone, 1:1, was used. The stock solution was used for derivatization or diluted to obtain on-scale GLC responses for non-derivatized pesticides.

Pesticide Derivatization

1. Perfluoroacylation method (12).

In the 15 ml culture tube, add 200 µg pesticide, 2 ml hexane, 50 µl pyridine, 50 µl pentafluoropropionic anhydride (PFPA). Mix well and allow reaction to proceed for one hr at room temperature. Add 3 ml phosphate buffer pH 7 and mix on Maxi-Mix to stop reaction. Add 3 ml hexane, 50 µl acetonitrile, and mix again. After separation of aqueous and organic phases, remove the bottom layer of aqueous phase with a disposable pipet and discard. Add 2 ml distilled water, mix and aspirate bottom layer; repeat washing two more times. Dry the hexane solution with sodium sulfate. Make final volume to 10 ml with hexane and adjust concentration for on-scale GLC response.

2. Pentafluorobenzoylation (20).

2.1. Pyridine System. In 15 ml culture tube, add 200 µg pesticide, 4 ml benzene, 10 µl of 0.1% pyridine in benzene, 2-5 µl of neat pentafluorobenzoylchloride (PFBC). Mix well and allow reaction to proceed for 30 min by refluxing in dry bath (95°C). Add 4 ml of pH 7 phosphate buffer and mix. After separation of aqueous and organic phases, remove the bottom layer of aqueous phase. Add 4 ml distilled water, mix and aspirate bottom layer; repeat washing two more times. Dry the organic solution with sodium sulfate. Make final volume to 10 ml and adjust concentration for on-scale GLC response.

2.2. Aqueous Sodium Hydroxide System (0.1 M). In 15 ml culture tube, add 200 μg pesticide, 4 ml 0.1 M NaOH, 2 μl PFBC. Mix well and allow reaction to proceed for 30 min by heating in a dry bath (95°C). After the reaction, add 4 ml of benzene to extract derivatized compound from aqueous phase. This was done in the same way as described in the pyridine system.

3. The derivatization of pesticides with the following compounds was similar to the pentafluorobenzoylation procedure (pyridine system) except that 10 μl neat pyridine was used: acetic acid N-hydroxy succinimide ester (AAHSE), N-methyl-bis-(heptafluorobutyryl) amide (MHFBA), N-methyl-bis-(trifluoroacetamide) (MBTFA), and 9-chloromethyl-anthracene (CMA)

9-chloromethyl-anthracene was synthesized by refluxing 9-anthracene-methanol with thionyl chloride in benzene for 3 hr (21).

4. Pentafluorobenzylation (17). In 15 ml culture tube, add 200 μg pesticide, 2 ml acetone, 1 ml pH 12 buffer, 10 μl α-bromo-2,3,4,5,6-pentafluorotoluene (BPFT). Mix well and allow reaction to proceed for 2 hr by refluxing in dry bath (100°C). Add 2 ml benzene and mix by shaking. After separation of aqueous and organic phases, remove the aqueous phase and

wash the organic phase twice with distilled water as previously described.

5. O-(2,3,4,5,6 Pentafluorobenzyl) hydroxylamine (PFBHA) derivatization (19). Make a stock solution of the reagent, PFBHA, in pyridine to contain 50 mg per ml.

In 15 ml culture tube, add 200 µg pesticide in 200 µl benzene. Remove benzene under a stream of N_2. Add 0.2 ml of the PFBHA stock solution. Vortex the tube to assure contact with the pesticide, stopper and heat in a dry bath at 85°C for half an hour. Remove the pyridine with a stream of N_2. Add one ml of hexane, mix, follow with 1 ml of distilled water. Shake the tube and allow the phases to separate. Transfer most of the hexane layer to another tube using a Pasteur pipet, and add anhydrous sodium sulfate to dry the hexane before GLC analysis.

6. Diazomethane derivatization (18). Pipet a benzene solution of an appropriately diluted pesticide standard into a 15 ml culture tube. Remove benzene under a stream of N_2. Add one ml of diazomethane reagent and let stand 10 min with occasional shaking. Evaporate solvent with N_2 and add 5 ml of benzene for GLC analysis.

7. Halogenated benzene-sulfonate formation (13). Reflux soil extract of pesticide with 2 mg of 2,5-dichlorobenzenesulfonyl chloride in 2 ml of acetone and 50 µl pyridine for 30 minutes. The reaction solution is cooled and 5 ml of hexane and 2 ml buffer pH7 are added. Shake well and separate the organic phase. Wash the organic phase three times with 2 ml

distilled water each time. Dry the organic phase with sodium sulfate and adjust volume for GLC analysis.

Preparation of Spiked Soil Samples and Extraction Procedure

Spike triplicate samples of 100 g (10% moisture) at 0, 0.01, 0.10, 1 and 10 ppm of pesticides and extract by Soxhlet extractor with 300 ml 1:1 benzene/methanol for 12 hours. Evaporate benzene/methanol extract by Kuderna-Danish evaporator with repetitive additions of benzene to eliminate methanol and water. Dilute the samples with benzene to obtain appropriate volume for direct GLC analysis. For further silica gel column cleanup, evaporate benzene almost to dryness and take the residue up in 1 ml hexane.

As an alternative procedure, tumble or shake one hundred grams of each soil sample for one hour with 300 ml of benzene, acetone or benzene/methanol (1:1), filter, evaporate to dryness on hot plate (50°C) under nitrogen, and take the residue up in 1 ml hexane.

Polytron Ultrasonic Extraction Procedures

Spike air-dried sandy soil samples of 50 g at 0, 0.01, and 0.10 ppm of pesticides and extract with the Polytron. The generator which is immersed in the soil sample is equipped with a saw-tooth cutting head. To the soil sample add 100 ml of benzene/methanol (1:1), unless stated otherwise. With the Polytron generator immersed in the sample, extract the soil twice for 60 sec. at maximum power unless otherwise noted. Filter the extracted soil and solvent through Whatman No. 42

filter paper. Rinse the filter paper and the soil twice with 50 ml of the extracting solvent. Evaporate combined filtrate to dryness under a stream of N_2 at room temperature (for captan, folpet, Difolatan, and methomyl), or by Kuderna-Danish evaporator, chromatograph the residue on the silica gel column (as below) and analyze gas chromatographically.

Silica Gel Column Chromatography

Transfer the hexane solutions of soil extracts to silica gel chromatographic columns. Prepare the column by packing 16 g of silica gel topped with 14 g of reagent grade Na_2SO_4. Add one ml of soil extract (equivalent to 100 g of soil) to the top of the bed with a disposable pipet. Wash the container with 2, 3 and 4 ml of hexane and deliver to the column respectively. Elute the column successively with 50 ml of the following solvent systems: (1) hexane, (2) 60% benzene in hexane, (3) 5% acetonitrile in benzene, (4) 10% acetonitrile in benzene, (5) 50% acetonitrile in benzene, and (6) acetonitrile. Evaporate these fractions and adjust to volume with benzene for GLC analysis.

Results and Discussion

Screening of pesticides for GLC responses

1. Electron capture detector. Results for fourteen halogenated pesticides on five GLC columns which gave relatively good responses are presented in Tables 2, 3, 4, 5 and 6. Gas chromatograms of these compounds on three GLC columns are illustrated by Figures 1 a, b, and c. Their analytical curves for quantitation of soil samples are presented in Figures 2-7.

Retention times and peak areas of fourteen halogenated pesticides relative to aldrin (RRT and RPA respectively; see footnotes Table 2) on a 10% DC-200 column are presented in Table 2. All pesticides are presented according to their elution orders from lowest to highest RRTs. Multiple peaks of Avadex indicated the presence of impurities in the originally furnished pesticide standard. A subsequently furnished standard produced only a single GLC peak (Tables 4, 5 and 6). Overlapping peaks on 10% DC-200 (Table 2) are: simazine and atrazine; captan and folpet; endosulfan and Perthane. Their decreasing degree of sensitivities (from highest RPA to lowest RPA) are: trifluralin, endosulfan, PCNB, Avadex, bromacil, methoxychlor, Perthane, Difolatan, chlorobenzilate, folpet, captan, atrazine, simazine and CIPC.

RRTs and RPAs of fourteen halogenated pesticides on 10% QF-1 are presented in Table 3. Overlapping peaks are: PCNB,

Table 2. Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 10% DC-200, 195°C (Att. = 5×10^{-9}, N_2 flows = 60 ml/min. Packard 7820, EC).

Pesticide	RRT[1]	RPA[2]	Analytical Curve (Figure No.)
Avadex	0.23, 0.29 0.37, 0.39, 0.57	0.500	—
CIPC	0.29	0.002	4
Trifluralin	0.34	2.300	5
Simazine	0.39	0.015	—
Atrazine	0.40	0.017	—
PCNB	0.48	0.550	7
Bromacil	0.92	0.302	5
Captan	1.18	0.050	—
Folpet	1.19	0.055	—
Endosulfan[3]	1.50, 2.03	0.570, 0.085	7
Perthane	1.98	0.101	4
Chlorobenzilate	2.36	0.078	4
Difolatan	3.11	0.088	—
Methoxychlor	4.60	0.240	7
Aldrin	1 (12.5 min)	1.000	—

[1] Computed as retention time relative to aldrin.

[2] $\text{RPA} = \dfrac{\text{peak area of pesticide/amount of pesticide}}{\text{peak area of aldrin/amount of aldrin}}$

[3] Only major peak of endosulfan was used for analytical curve construction.

Note: Major peaks are indicated by underlining.

Table 3. Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 10% QF-1, 195°C (Att. = 1 x 10^{-9}, N_2 flow = 60 ml/min. Packard 7820, EC).

Pesticide	RRT[1]	RPA[2]
Avadex	0.50	0.017
CIPC	0.63	0.001
PCNB	0.77	0.039
Simazine	0.79	0.002
Atrazine	0.75	0.013
Trifluralin	1.90	0.394
Perthane	1.69	0.065
Endosulfan	2.31, 4.00	0.390, 0.185
Folpet	2.92	0.292
Bromacil	3.08	0.358
Captan	3.23	0.306
Chlorobenzilate	3.54	0.083
Methoxychlor	5.00	0.173
Difolatan	8.17	0.167
Aldrin	1 (2.5 min)	1.000

[1&2] See footnotes of Table 2.

Note: Major peaks are indicated by underlining.

Table 4. Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 4% SE-30/6% OV-210, 205°C (Att. = 2 x 10^2, N_2 flow = 67 ml/min, Tracor 222, EC).

Pesticide	RRT[1]	RPA[2]	Analytical Curve (Figure No.)
CIPC	0.38	0.004	—
Avadex	0.46	0.019	2
Simazine	0.52	0.004	6
Atrazine	0.54	0.007	6
Trifluralin	0.54	0.247	—
PCNB	0.62	0.430	—
Bromacil	1.48	0.230	—
Endosulfan	1.75, 2.56	0.700, 0.210	—
Folpet	1.76	0.136	5
Captan	1.76	0.105	5
Perthane	1.91	0.008	—
Chlorobenzilate	2.63	0.025	—
Methoxychlor	4.30	0.242	—
Difolatan	4.40	0.105	5
Aldrin	1 (7.5 min)	1.000	—

[1&2] See footnotes of Table 2.

Note: Major peaks are indicated by underlining.

Table 5. Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 1.5% OV-17/1.95% OV-210, 205°C (Att. 2 x 10^{2}, N_2 flow = 53 ml/min. Tracor 222, EC).

Pesticide	RRT[1]	RPA[2]
Trifluralin	0.45	0.204
CIPC	0.45	0.002
Avadex	0.53	0.031
PCNB	0.70	0.420
Atrazine	0.70	0.009
Simazine	0.70	0.008
Chlorobenzilate	1.55, 3.32	0.012, 0.019
Endosulfan	1.97, 3.58	0.660, 0.130
Folpet	2.70	0.070
Captan	2.70	0.071
Perthane	2.70	0.013
Bromacil	3.25	0.061
Difolatan	7.35	0.026
Methoxychlor	7.80	0.139
Aldrin	1 (2.8 min)	1.000

[1&2] See footnotes of Table 2.

Note: Major peaks are indicated by underlining.

Table 6. Retention times and response values relative to aldrin of fourteen EC-sensitive pesticides on 5% OV-210, 205°C (Att. 2 x 10^2, N_2 flow = 53 ml/min. Tracor 222, EC).

Pesticide	RRT[1]	RPA[2]
Avadex	0.59	0.014
CIPC	0.69	0.002
Atrazine	0.85	0.008
Simazine	0.85	0.008
PCNB	0.88	0.680
Trifluralin	1.05	0.230
Perthane	1.65	0.008
Chlorobenzilate	1.78, 3.25	0.011, 0.029
Endosulfan	2.22, 3.75	0.860, 0.180
Folpet	3.00	0.039
Captan	3.16	0.649
Bromacil	3.69	0.129
Methoxychlor	4.60	0.108
Difolatan	7.10	0.009
Aldrin	1 (2.3 min)	1.000

[1&2] See footnotes of Table 2.

Note: Major peaks are indicated by underlining.

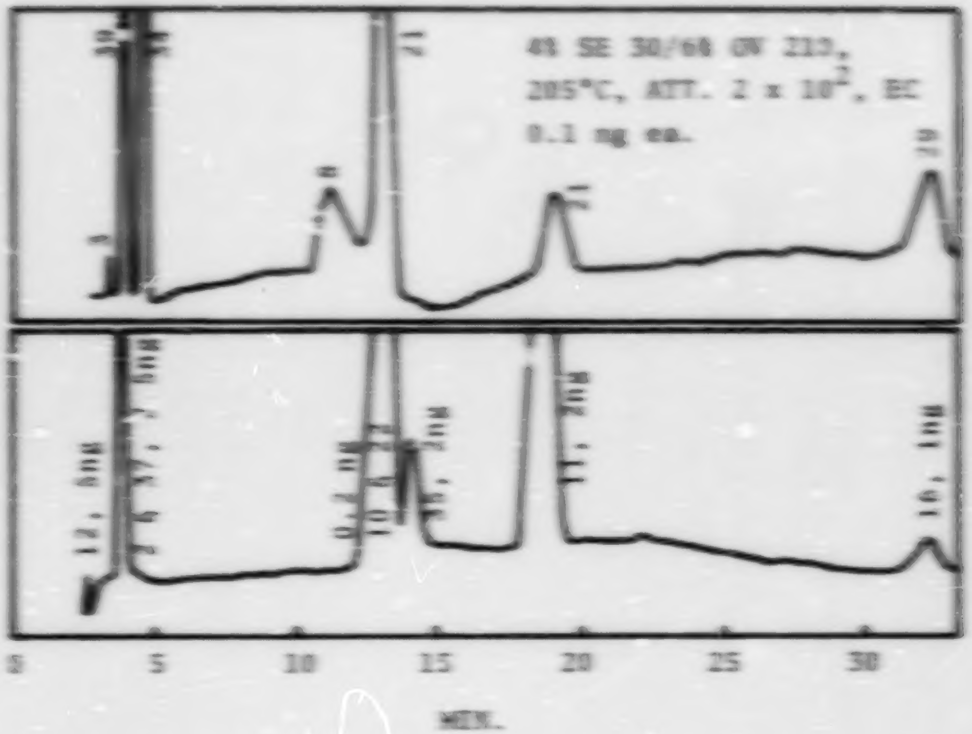


FIGURE 1 a

Chromatograms of 14 EC-sensitive pesticides. Pesticide mixtures were grouped to prevent peak coincidence; numbers correspond to pesticides listed in Table 1.

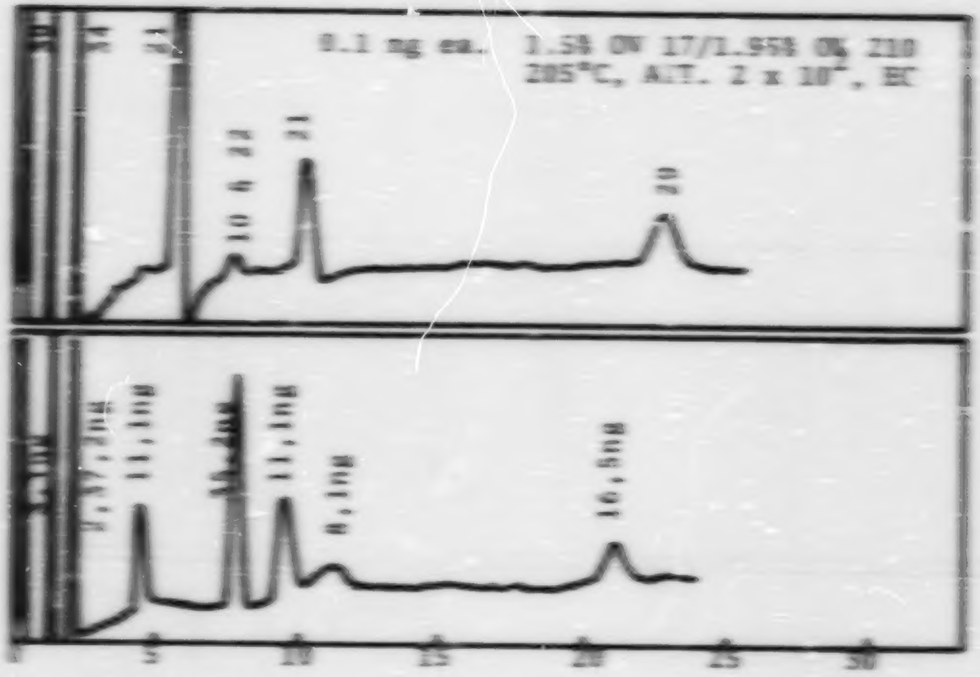


FIGURE 1 b

Chromatograms of 14 EC-sensitive pesticides on 1.5% OV-17/ 1.95% OV-210 column.

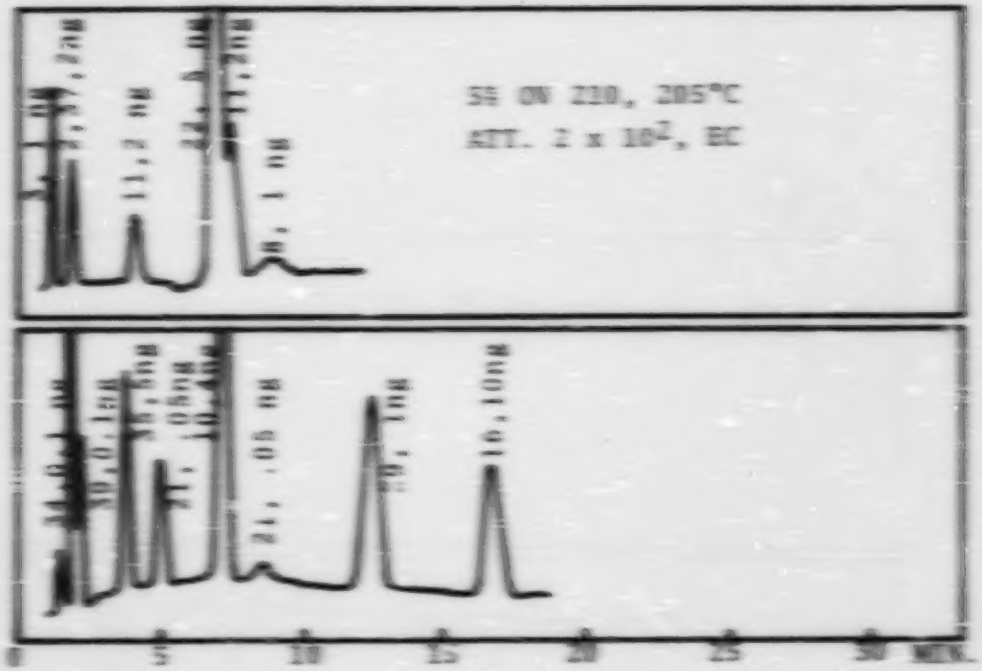


FIGURE 1 c

Chromatograms of 14 EC-sensitive pesticides.

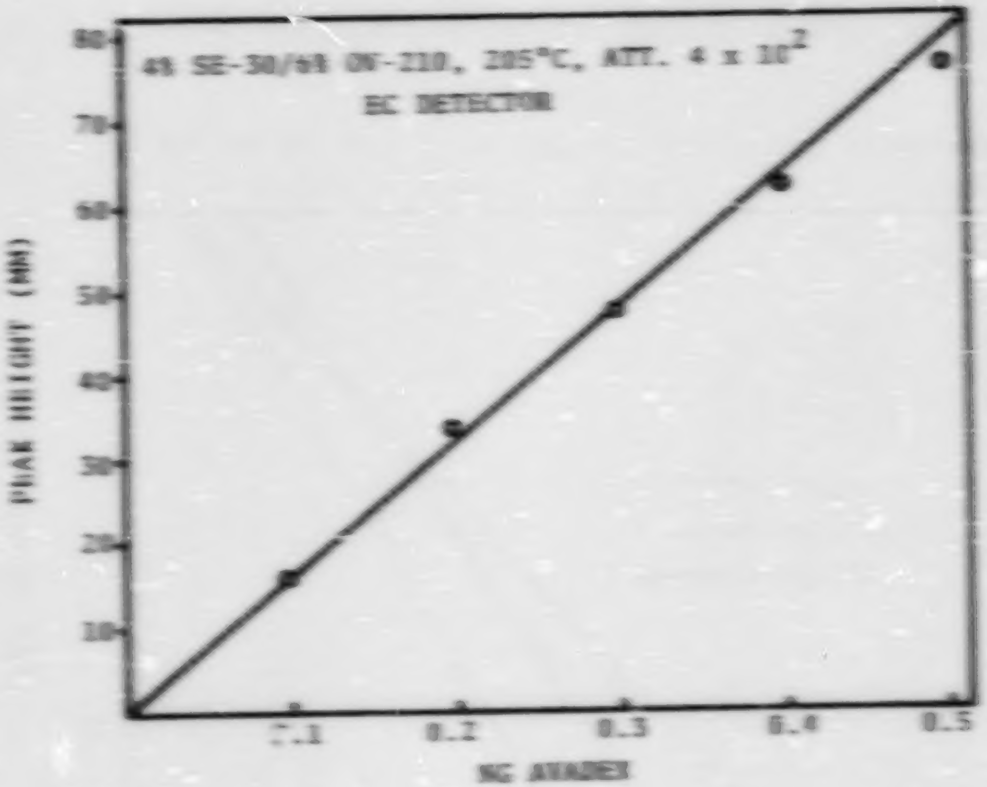


FIGURE 2

Analytical curve of Avadex.

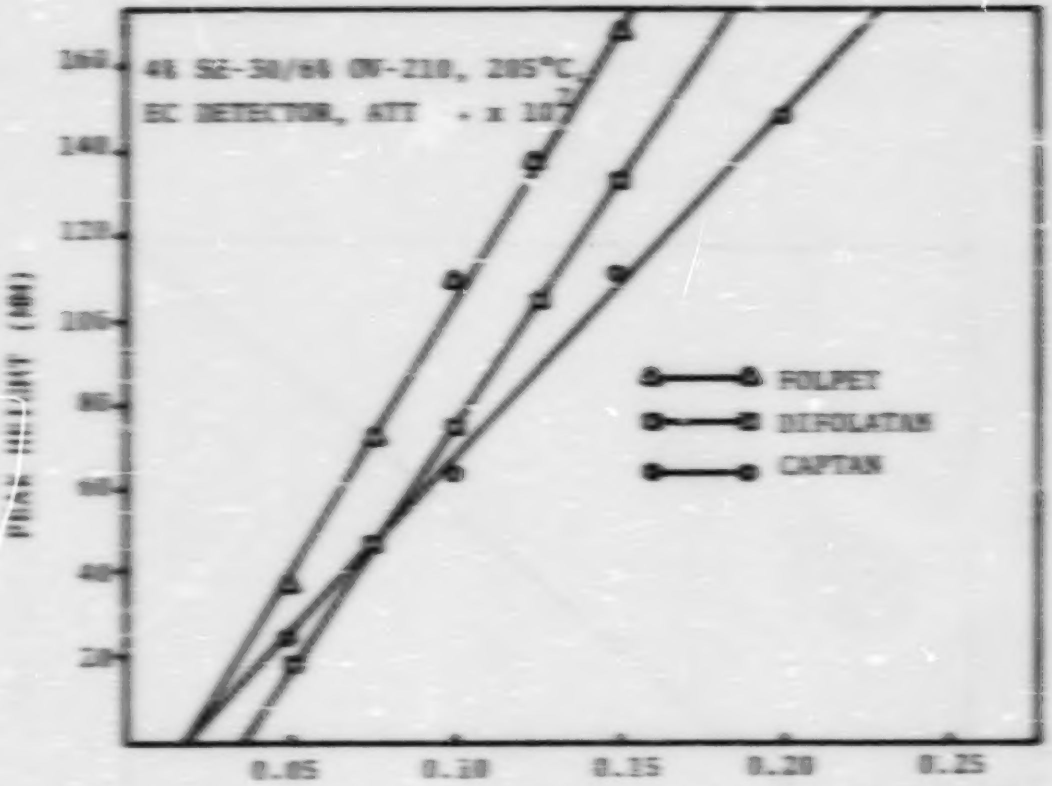


FIGURE 3

Analytical curves of Captan, Folpet and Difolatan.

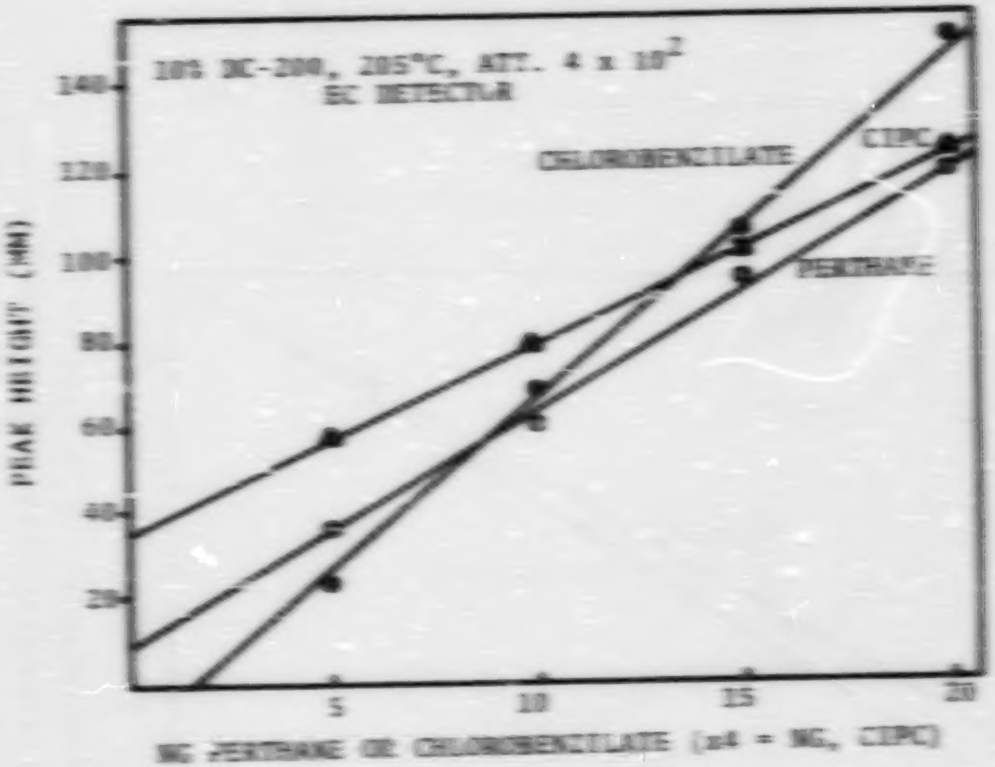


FIGURE 4

Analytical curves of Perthane, Chlorobenzilate and CIPC.

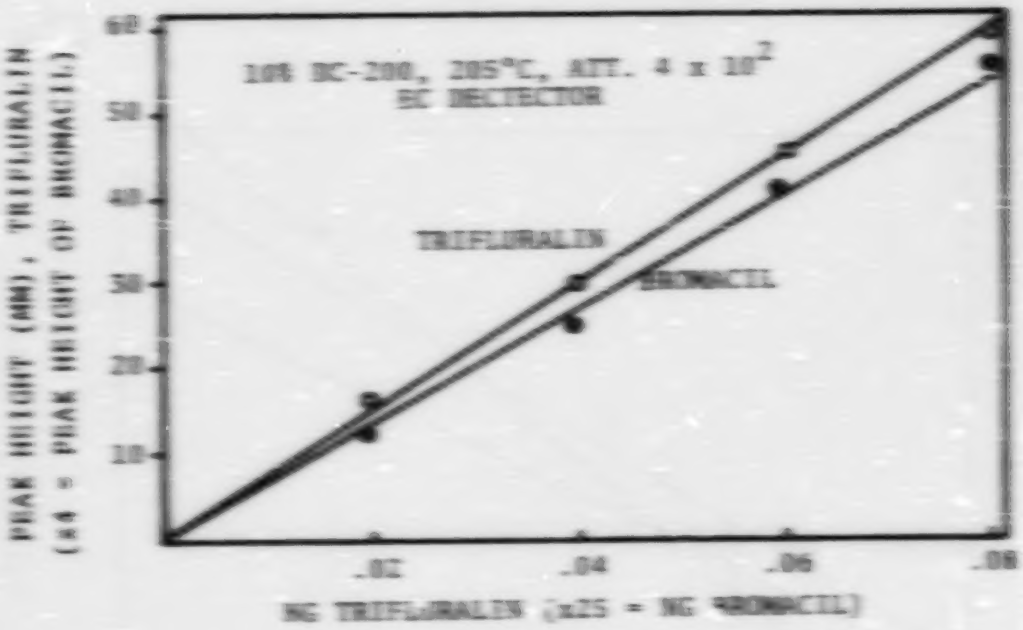


FIGURE 5

Analytical curves of Trifluralin and Bromacil.

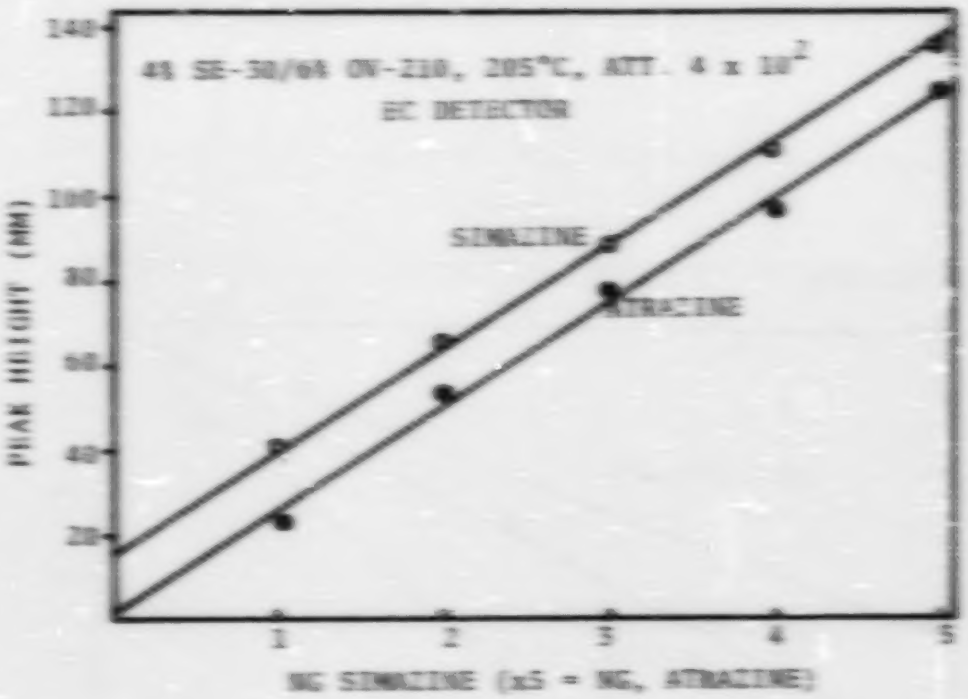


FIGURE 6

Analytical curves of Simazine and Atrazine.

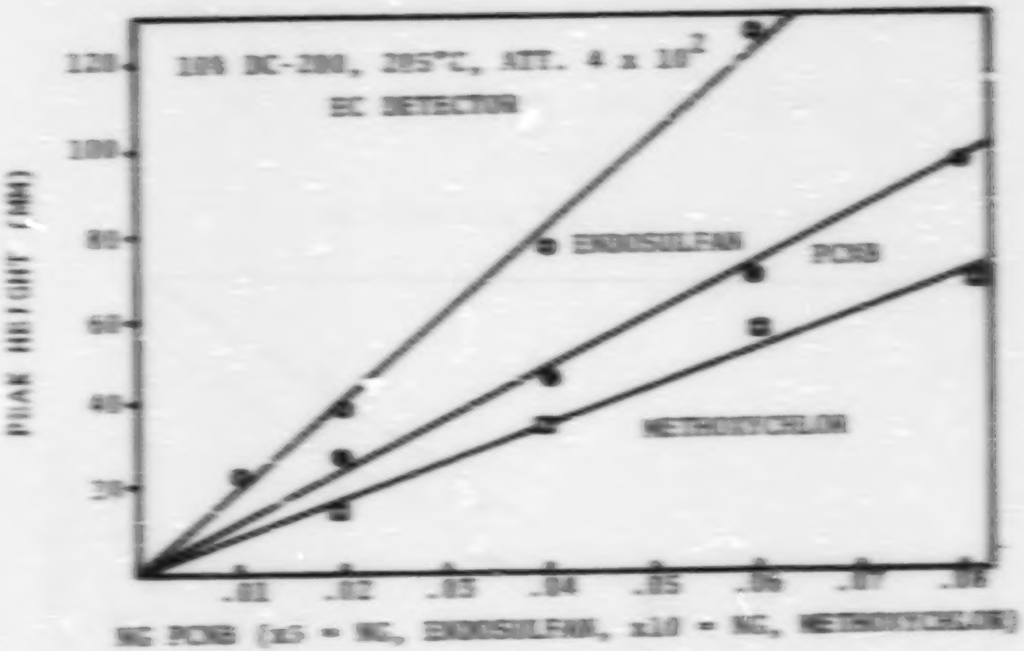


FIGURE 7

Analytical curves of PCNB, Endosulfan and Methoxychlor.

simazine, atrazine; folpet and bromacil. Their decreasing degree of sensitivities are: trifluralin, endosulfan, bromacil, captan, folpet, methoxychlor, Difolatan, chlorobenzilate, Perthane PCNB, Avadex, atrazine, simazine and CIPC. Table 4 presents RRTs and RPAs of these compounds on the 4% SE 30/6% OV-210 column. Overlapping peaks are: simazine (37), atrazine (2) and trifluralin (39); endosulfan (21), folpet (22) and captan (10); chlorobenzilate (11) and endosulfan (21), methoxychlor (29) and Difolatan (16) (see Figure 1 a for chromatograms). Their decreasing degree of sensitivities are: endosulfan, PCNB, trifluralin, methoxychlor, bromacil, folpet, captan, Difolatan, chlorobenzilate, Avadex, Perthane, atrazine, simazine and CIPC.

RRTs and RPAs of fourteen halogenated pesticides on 1.5% OV-17/1.95% OV-210 are presented in Table 5. Overlapping peaks are: trifluralin (39) and Avadex (3); PCNB (34), atrazine (2) and simazine (37); folpet (22), captan (10) and Perthane (35) (see Figure 1 b for chromatograms). Their decreasing degree of sensitivities are: endosulfan, PCNB, trifluralin, methoxychlor, captan, folpet, bromacil, Avadex, Difolatan, chlorobenzilate, Perthane, atrazine, simazine and CIPC.

Table 6 presents RRTs and RPAs of these compounds on the 5% OV-210 column. Overlapping peaks are: atrazine (2), simazine (37), PCNB (34), and trifluralin (39); folpet (22) and captan (10); endosulfan (21) and bromacil (8) (Figure 1 c). Their decreasing degree of sensitivities are: endosulfan, PCNB, trifluralin, bromacil, methoxychlor, captan, folpet, chlorobenzilate, Avadex, Difolatan, atrazine, simazine, Perthane and CIPC.

From Figure 1 a, the overlapping peaks of atrazine (2), simazine (37) and trifluralin (39) on the 4% SE 30/6% OV-210 column could be separated by the 1.5% OV-17/1.95% OV-210 column (Figure 1 b). None of the columns under investigation could be used to separate atrazine (2) and simazine (37). The best column for the separation of captan (10), folpet (22) and endosulfan (21) was the 5% OV-210 column (Figure 1 c). The overlapping peaks between chlorobenzilate (11) and endosulfan (21), methoxychlor (29) and Difolatan on the 4% SE 30/6% OV-210 column (Figure 1 a) could be separated by 5% OV-210 (Figure 1c).

Most of the analytical curves for the halogenated pesticides (Figure 2-7) exhibit good linearity and they intercept the point of origin except for folpet, Difolatan and captan (Figure 3), CIPC, chlorobenzilate and Perthane (Figure 4) and simazine (Figure 6), suggesting minor loss of these pesticides in the GLC system. This might be due to some degradation of these pesticides on the GLC column under elevated temperature or loss by absorption on GLC liquid phases. However, quantitation of these pesticides was still possible and fairly accurate results could be obtained because consistent and linear analytical curves were observed.

Unsatisfactory results were obtained for most nitrogen-containing pesticides and carbamate type compounds with the EC detector. Except for the tailing peak of bromacil and low sensitivity of CIPC, those pesticides giving moderately sensitive peaks were atrazine, bromacil, Difolatan and simazine (Tables 2-6). Zectran gave inconsistently unacceptable low sensitivity.

Those pesticides producing inconsistent multiple peaks or no responses were amitrole, benomyl, monuron, DNOC, DNBP, dicamba, Landrins (2,3,5 & 3,4,5), Zectran, IPC, bux and carbofuran.

Theoretical plates were obtined from aldrin peaks for five tested GLC columns (Table 7). 4% SE 30/6% OV-210 gave the highest theoretical plates; therefore it exhibited superior separations (Figure 1 a).

2. Flame photometric detector (phosphorous mode). Retention times and peak areas of organophosphate (OP) pesticides relative to parathion on five GLC columns are presented in Tables 8-11. They are presented according to their elution order (from lowest to highest RRTs). Analytical curves are presented in Figures 8-13.

Table 8 presents RRTs and RPAs of eight OP pesticides on 10% DC-200 and 10% QF-1 columns. The overlapping peaks on 10% DC-200 are phorate, Azodrin and demeton; on 10% QF-1 methyl parathion and malathion do not separate. No GLC peaks were obtained from the injection of Azodrin and azinphos methyl on 10% QF-1. Their decreasing sensitivities on 10% DC-200 are: azinphos methyl, parathion, methyl paration, phorate, malathion, azinphos ethyl, demeton, and Azodrin, on 10% QF-1: parathion, malathion, phorate, methyl parathion, azinphos ethyl, demeton, azinphos methyl, and Azodrin.

Table 9 presents RRTs and RPAs of twelve OP pesticides on a 4% SE 30/6% OV-210 column. Overlapping occurs for dimethoate and Azodrin; methyl parathion and malathion; parathion and phosphamidon. Their decreasing degree of sensitivities are: diaz-

Table 7. Computed efficiencies of five GLC columns (Tracor 222, tritium foil EC detector, column temperature = 205°C. Att. 4 x 10^2).

Column	Efficiency[1] (Theoretical Plates)	N_2 Flow (mℓ/min)
4% SE-30/6% OV-210	2700	67
1.5% OV-17/1.95% OV-210	2506	53
10% DC-200	1956	67
5% OV-210	1849	53
10% QF 1[2]	676	60

[1] Efficiency computed from total retention and base width of aldrin peak.

[2] Result was obtained from Packard 7820, EC.

Table 8. Retention times and response values relative to parathion of eight organophosphate pesticides on two GLC columns (Att. 8 x 10^{-8}, Varian 1200, FPD-P).

Pesticide	10% DC-200: 200° C, 50 ml/min		10% QF-1: 200° C, 50 ml/min	
	RRT[1]	RPA[2]	RRT[1]	RPA[2]
Phorate	0.36	0.63	0.29	0.53
Azodrin	0.37	0.01	no peak[3]	—
Demeton	0.39	0.11	0.34	0.11
Methyl parathion	0.68	0.71	0.78	0.42
Malathion	0.89	9.26	0.76	0.64
Parathion	1 (4.4 min)	1.00	1 (3.8 min)	1.00
Azinphos methyl	5.45	1.06	no peak[3]	—
Azinphos ethyl	7.25	0.20	6.10	0.29

[1] Computed as retention time relative to parathion.

[2] $\mathrm{RPA} = \dfrac{\text{peak area of pesticide/amount of pesticide}}{\text{peak area of parathion/amount of parathion}}$

[3] No responses when 1 μg of pesticides were injected.

Table 9. Retention times and response values relative to parathion of twelve organophosphate pesticides on 4% SE-30/6% OV-210, 200° C (Att. 8 x 10^{-8}, N_2 flow = 50 ml/min. Varian 1200, FPD-P).

Pesticide	RRT[1]	RPA[2]	Analytical Curve (Figure No.)[3]
Demeton	0.17, 0.32	0.300, 0.500	(11)
Phorate	0.26	1.500	(11)
Diazinon	0.29	1.840	10
Dimethoate	0.48	0.414	9
Azodrin	0.50	0.100	8
Dursban	0.64	1.700	9
Methyl parathion	0.77	0.696	(13)
Malathion	0.82	0.555	(12)
Parathion	1.00 (4.9 min)	1.000	(12)
Phosphamidon	0.74, 1.045	0.047, 0.285	9
Azinphos methyl	5.26	0.278	(13)
Azinphos ethyl	6.50	0.370	(13)

[1&2] See footnotes of Table 8.

[3] Parentheses indicate other GLC conditons were used; see these conditions from respective figures.

Table 10. Retention times and response values relative to parathion of twelve organophosphate pesticides on 1.5% OV-17/1.95% OV-210, 200° C (Att. 8 x 10^{-8}, N_2 flow = 50 ml/min. Varian 1200, FPD-P).

Pesticide	RRT[1]	RPA[2]
Demeton	0.16, 0.32	0.395, 0.762
Phorate	0.24	1.240
Diazinon	0.32	0.620
Dimethoate	0.54	0.830
Azodrin	0.59	0.162
Phosphamidon	0.68, 0.97	0.034, 0.149
Dursban	0.76	1.270
Methyl parathion	0.77	1.490
Malathion	0.88	1.030
Parathion	1 (2.6 min)	1.000
Azinphos methyl	9.65	1.070
Azinphos ethyl	12.00	0.780

[1&2] See footnotes of Table 8.

Table 11. Retention times and response values relative to parathion of twelve organophosphate pesticides on 5% OV-210, 200° C (Att. 32 x 10^{-9}, N_2 flow = 50 ml/min. Varian 1200, FPD-P).

Pesticide	RRT[1]	RPA[2]
Demeton	0.12, 0.31	0.202, 0.780
Phorate	0.17	0.500
Diazinon	0.19	1.610
Dursban	0.43	1.285
Dimethoate	0.52	0.450
Azodrin	0.67	0.083
Malathion	0.74	0.525
Methyl parathion	0.79	0.875
Parathion	1 (3.04 min)	1.000
Phosphamidon	1.32	0.136
Azinphos methyl	5.10	0.233
Azinphos ethyl	6.20	0.212

[1&2] See footnotes of Table 8.

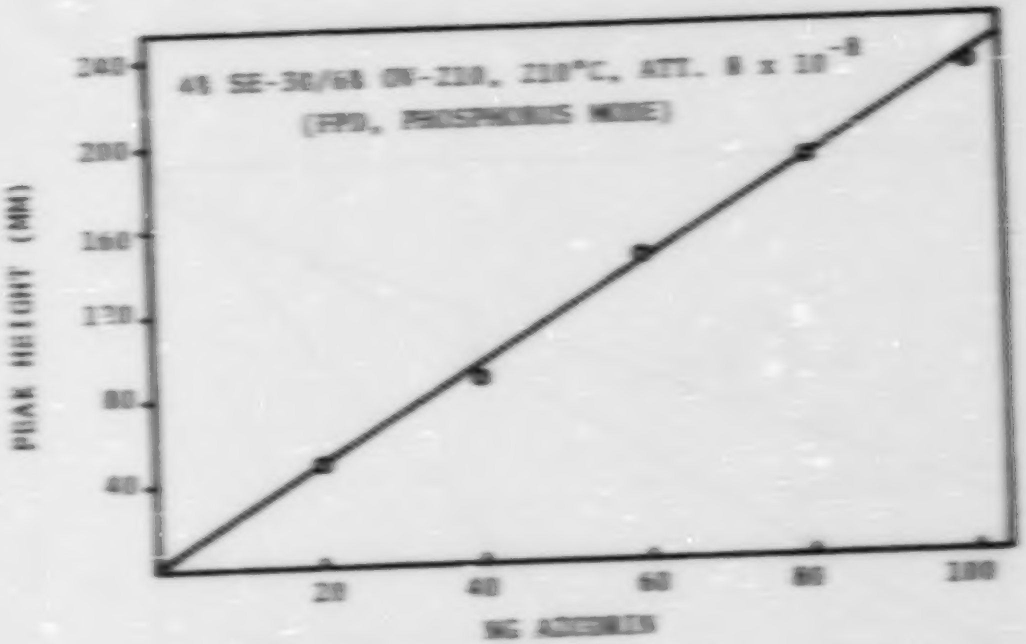


FIGURE 8

Analytical curve of Azodrin.

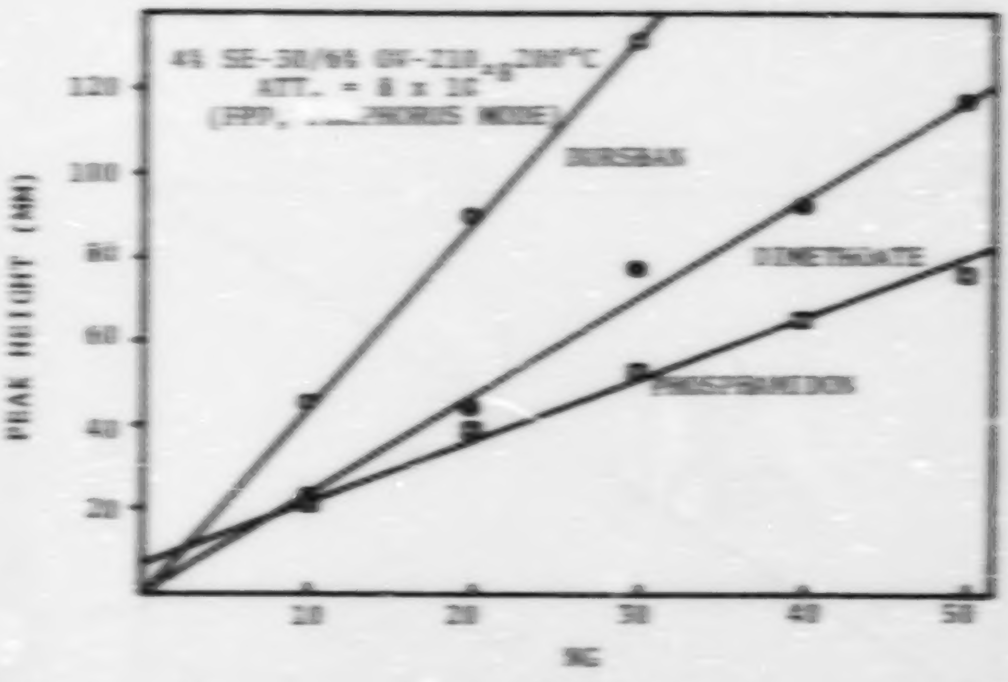


FIGURE 3

Analytical curves of Dursban, Dimethoate and Phosphamidon.

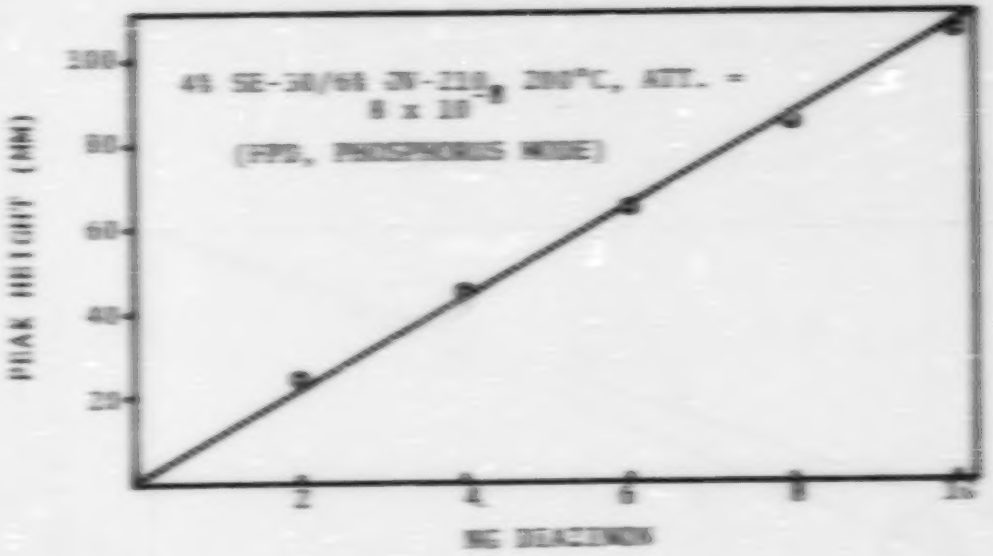


FIGURE 10

Analytical curve of Diazinon

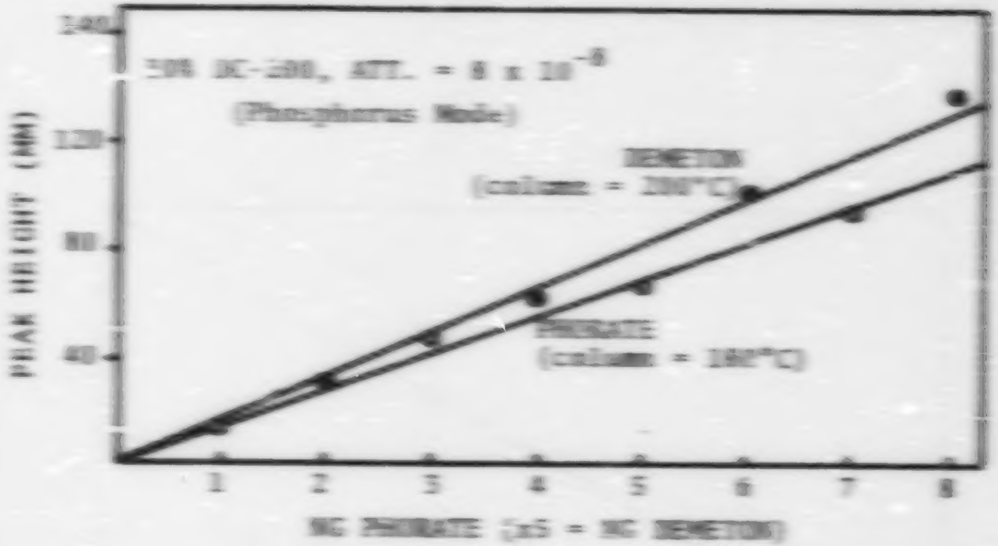


FIGURE 11

Analytical curve of phorate and demeton.

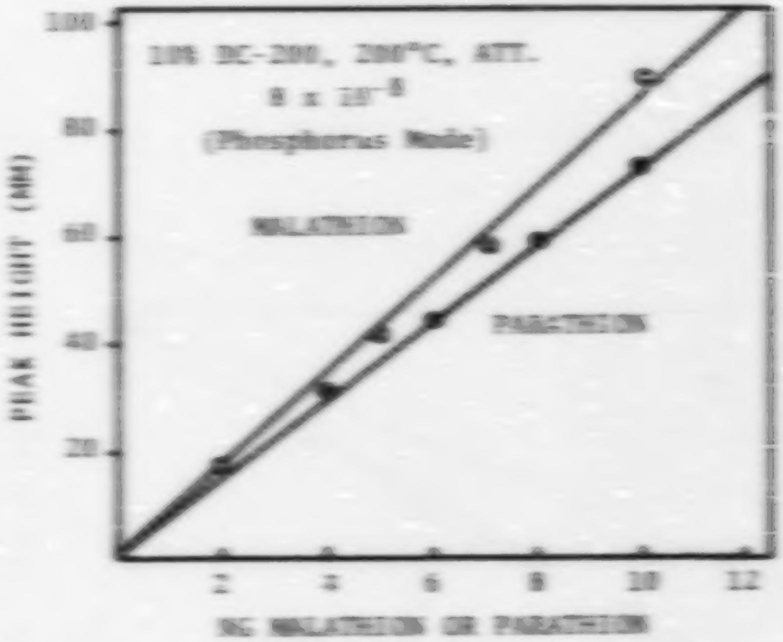


FIGURE 12

Analytical curves of malathion and parathion.

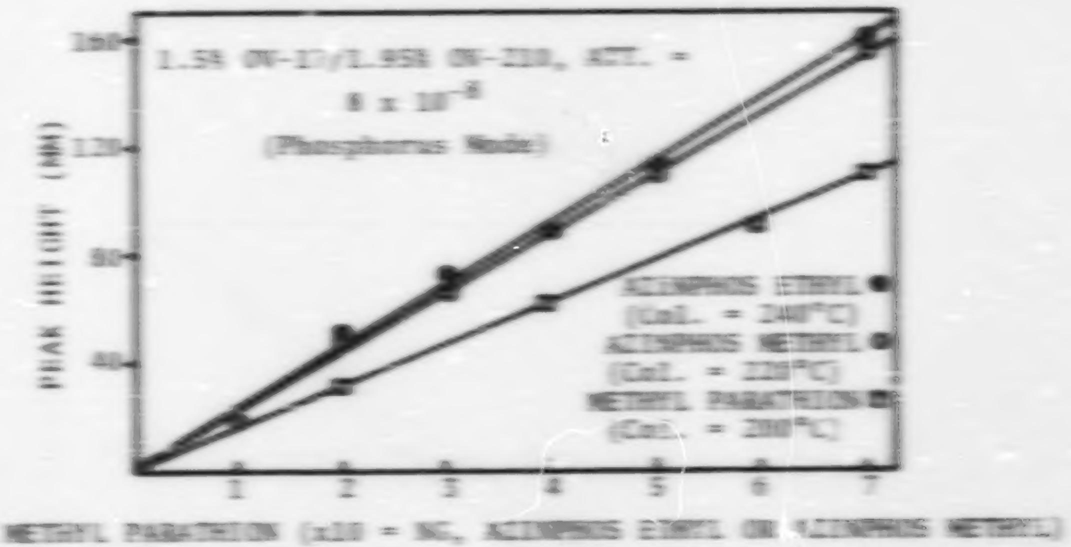


FIGURE 13

Analytical curves of Azinphos Ethyl, Azinphos Methyl and Methyl Parathion inon, Dursban, phorate, parathion, methyl parathion, malathion, demeton, dimethoate, azinphos ethyl, phosphamidon, azinphos methyl and Azodrin.

Table 10 presents RRTs and RPAs of twelve OP pesticides on 1.5% OV-17/1.95% OV-210 column. The overlapping peaks are: demeton and diazinon; dimethoate and Azodrin; Dursban and methyl parathion. Their decreasing degree of sensitivities are: methyl parathion, Dursban, phorate, azinphos methyl, malathion, parathion, dimethoate, azinphos ethyl, demeton, diazinon, Azodrin and phosphamidon.

Table 11 presents RRTs and RPAs of twelve OP pesticides on 5% OV-210 column. The overlapping peaks are: phorate and diazinon; malathion and methyl parathion. Their decreasing degree of sensitivities are: diazinon, Dursban, parathion, methyl parathion, demeton, malathion, phorate, dimethoate, azinphos methyl, azinphos ethyl, phosphamidon and Azodrin.

It was uncertain whether the minor peak for phosphamidon was an impurity or derived from degradation on the GLC column (Tables 9-10). However, the reproducibility of the major peak was good and only this peak was used for recovery computation. All of the analytical curves for the organophosphate pesticides (Figures 8-13) exhibit good linearity, and intercept the point of origin except for phosphamidon (Figure 9), suggesting increasing loss of phosphamidon when larger amounts were injected.

3. Hall electrolytic conductivity detector. Table 12 presents retention times and response values relative to atrazine of some nitrogen-containing pesticides. Most of the

tested pesticides did not give acceptable GLC peaks, which could be due to the unsuitable GLC column or thermal decomposition of pesticides. 28% Pennwalt 223/4% KOH and 5% Poly A-135 on 80/100 mesh Gas-Chrom R, which are used primarily for the separation of simple amines or anilines, were tested for these nitrogen-containing pesticides. Unfortunately, no GLC peaks from any of the tested pesticides were observed (column temperature 160°-180°C). It might be worthwhile to note that benomyl produced a peak which appeared immediately after the hexane solvent peak (5% SE-30 column). This peak might not be the intact benomyl peak because one would not expect it to have such a short retention time. It is assumed that benomyl may have undergone thermal decomposition to give butylisocyanate.

Some solvents such as ethylacetate, methylene chloride or other halogen-containing compounds would severely contaminate the Hall detection system and consequently create noisy baselines. Hexane seems to be the best solvent for this detector; however, it was observed that a periodic injection of pyridine (1 ul) corrected the noisy baseline. Pyridine probably neutralized the excess acids in the GLC system generated by repetitive sample injections.

Derivatization of pesticides

Derivatization of nitrogen-containing pesticides was attempted by using pentafluoropropionic anhydride (PFPA) (Tables 13-14) and 2,5-dichlorobenzenesulfonyl chloride (DBSC) (Table 15). Most of the pesticides which have a proton attached to the nitrogen and an aromatic structure gave positive results

Table 12. Retention times and response values relative to atrazine, of Nitrogen-containing pesticides on 5% SE-30 column, 180° C (Hall detector, att. = 1 x 10; furnace, 820° C; H_2 flow, 35 ml/min; cell flow, 0.5 ml/min; He flow, 67 ml/min; inlet, 225° C; transfer line, 260° C).

Pesticide	RRT	RPA	Remarks
Atrazine	1 (1.87 min)	1	9 mm^2/ng, 14% Full scale response
CIPC	0.76	0.206	
Carbofuran	1.00	0.035	
Simazine	1.05	4.200	
IPC	0.333	2.600	
Zectran	0.28, 1.38	0.48, 0.30	two peaks
DNOC (500 ng)			no peak
Temik (1 ug)			inconsistent tailing peak
Benomyl (1 ug)			no peak except enlarged solvent peak
Aradex (1 ug)			noisy multiple peaks
Bux (1 ug)			multiple tailing peaks
Amitrole (1 ug)			no peak
Azodrin (1 ug)			multiple peaks
Monuron (1 ug)			inconsistent tailing peaks

Table 13. Retention times and response values relative to aldrin of PFPA derivatized pesticides on two columns (Packard 7820, EC).

	10% DC-200; 195° C, 60 ml/min		10% QF-1; 195° C, 60 ml/min	
	Att. 3 x 10^{-9}		Att. 1 x 10^{-9}	
Pesticide	RRT[1]	RPA[2]	RRT[1]	RPA[2]
IPC	0.08	0.230	0.31	0.159
CIPC	0.16	0.088	0.38	0.0556
Simazine	0.26	0.640	0.62	0.346
Atrazine	0.28	0.240	0.62	0.236
Monuron	0.28	0.067	1.00	0.0727
Furadan	0.29	0.158	0.67	0.158
Bux	0.34, 0.41	0.167, 0.0206	0.58, 0.69	0.184, 0.0455
Zectran	0.38	0.0111	0.62	0.018
Aldrin	1 (13 min)	1.0000	1 (2.5 min)	1.600

[1&2] See footnotes of Table 2.

The pesticides which were also derivatized and produced no responses were Avadex Benomyl, Bromacil, Difolatan, Amitrole, Diquat, DNOC, Ferbam, Temik and Azodrin (PFPA of Azodrin was also checked by FPD, no response was observed).

Table 24. Retention times and response values relative to aldrin of PFPA derivatized pesticides on two columns (Tracor 222, EC, Att. 4 x 10^2, column at 195°C).

Pesticide	4% SE-30/6% OV-210 N_2 flow = 67 ml/min RRT[1]	 RPA[2]	1.5% OV-17/1.95% OV-210 N_2 flow = 55 ml/min RRT[1]	 RPA[2]
Aldrin	1.000	1.000	1.000	1.000
2,3,5-Landrin	0.350	0.063	0.270	0.218
3,4,5-Landrin	0.425	0.030	0.346	0.087
2-AB[3]	UD	—	—	—
DNBP	UD	—	—	—
Dicamba	UD	—	—	—

[1&2] See footnotes of Table 2.

[3] 2-AB = 2-aminobenzimidazole, a degradation product of benomyl.

UD = underivatizable.

Table 15. Retention times and response values relative to aldrin, of 2,5-dichlorobenzenesulfonyl chloride derivatized pesticides (Pyridine system) on two GLC columns (Tracor 222, EC, Att. 4 x 10^{2})

Pesticide	4% SE-30/6% OV-210 220° C, 60 ml/min		1.5% OV-17/1.95% OV-210 220° C, 60 ml/min	
	RRT[1]	RPA[2]	RRT[1]	RPA[2]
Carbofuran	0.50	0.013	3.75	0.024
Temik[3]	0.69	0.046	0.75	0.051
DNOC	0.75	0.400	0.75	0.700
Mesurol	1.00	0.067	0.50	0.050
Alpha-naphthol[4]	4.00	0.040	6.00	0.034
Bux	5.00, 6.00	0.028, 0.005	3.00, 4.00	0.025, 0.008
Aldrin	1 (1.25 min)	1.000	1 (0.63 min)	1.000

[1&2] See footnotes of Table 2.

[3] Similar results were obtained for methomyl.

[4] When carbaryl was derivatized in buffer pH 12 system peaks of RRT 4.0 and 0.69 appeared.

The pesticides which were also derivatized and produced no responses were Amitrole, Aradex, Benomyl, Azodrin, Simazine, Atrazine, Bromacil, CIPC, IPC, Zectran Sevin and Difolatan.

(Table 13-14). Linear analytical curves are presented in Figures 14 and 15. The thermal instability of N-methylcarbamates and their reaction with acylating agents were briefly discussed by Khalifa and Mumma (8). Damico and Benson (14) postulated that the proton attached to nitrogen is readily transferable to the phenolic oxygen when the carbamate is subjected to elevated temperatures or under mass spectroscopic conditions. When the proton attached to the nitrogen is replaced, the products have greater thermal stability (15).

Moye's original derivatization techniques (13) of DBSC with carbamates were modified by substituting the pH 12 buffer with a pyridine system (Table 15). This relatively high pH system may split the intact carbamates before they react with DBSC. From comparison of the results obtained from carbaryl and alpha-naphthol in these two reaction systems, one might be able to postulate the reaction mechanisms of carbaryl and other similar carbamates with DBSC. In the pH 12 buffer system, carbaryl was split into two reactive parts, namely alpha-naphthol and methylamine or methylisocyanate which subsequently reacted with DBSC to yield 1-naphthyl--2,5-dichlorobenzene sulfonamide (RRT = 4.00) and N-methyl 2,5-dichlorobenzene sulfonamide (RRT = 0.69) respectively (Table 15). In either the pH 12 or pyridine system, 1-naphthyl-2,5-dichlorobenzenesulfonate (RRT = 4.00) was obtained when alpha-naphthol was substituted for carbaryl. In the pyridine system, carbaryl is likely to stay intact because no GLC peaks were produced. However, DBSC derivatives of Temik (RRT = 0.69) and methomyl (RRT = 0.69) in the pyridine system

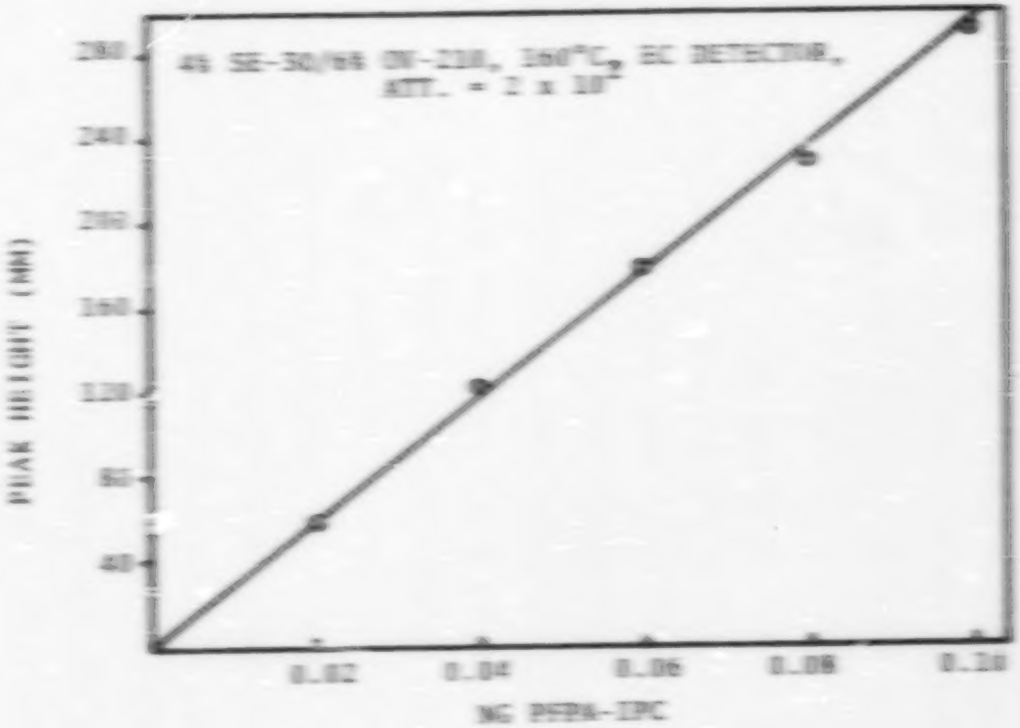


FIGURE 14

Analytical curve of PFPA-IPC.

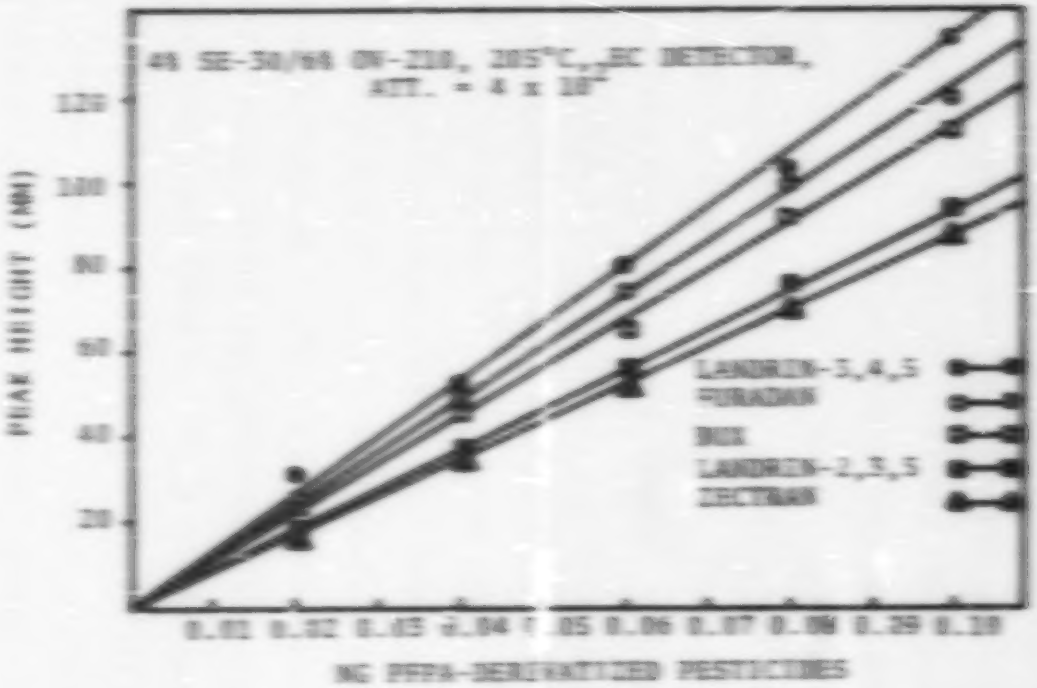


FIGURE 15

Analytical curves of Furadan, Bux, Zectran, Landrins (PFPA).

both gave identical RRT peaks, and they correspond to N-methyl-2,5-dichlorobenzene sulfonamide.

Retention times and response values relative to aldrin of pentafluorobenzoyl chloride (PFBC) derivatized pesticides in pyridine and 0.1 M NaOH systems are presented in Tables 16-18 and Tables 19-20 respectively. The best results obtained in the pyridine system were for amitrole, benomyl and monuron, although the resulting derivatives were far from perfect. One of the serious short-comings was the interference of excess PFBC which could not be eliminated by simple solvent partition. The PFBC GLC peak fused together with the solvent peak and caused serious tailing for about 15 min. Therefore, from Table 17, the short RRT (0.316) of amitrole was masked by excess PFBC, although it created no serious problem for the longer RRTs of benomyl (14.3) and monuron (2.98). A disadvantage of PFBC-amitrole was the tailing peak, which leads one to suspect its structural stability under normal GLC conditions. PFBC-benomyl seemed to be ideal for GLC detection except for its somewhat low sensitivity (RPA = 0.077) and long RRT (14.3). PFBC-monuron offered the best PFBC-derivative which could be applied to routine analyses. Attractive qualities were seen in the moderately long RRT (2.98) which separated it from most of the soil impurities and the good EC-GLC response (RPA = 0.73). The non-reactivity of PFBC with most soil impurities made it even more attractive for application to routine residue analysis. The linear analytical curves of PFBC-derivatives of amitrole, benomyl and monuron are presented in Figures 16-18.

Table 16. Retention times and response values relative to aldrin of PFBC derivatized pesticides (pyridine system) on 10% DC-200 column, 205°C (Att. = 4×10^{2}, N_2 flow = 80 ml/min. Tracor 222, EC).

Pesticide	RRT	RPA
Amitrole	0.188	1.960
Avadex	UD[2] (2.07, 0.37, 0.56)[1]	(0.033)[1]
Azodrin	1.00	0.018
Benomyl[3]	10.400	0.110
Bux	0.575, 0.690	0.004, 0.005
DNOC	UD[2]	—
Carbofuran	0.580	0.024
IPC	0.500	0.003
Monuron	1.310	1.07
Temik	UD[2]	—
Zectran	0.810	0.003

[1] Data represents original structure of avadex, only major peak (underlining) was computed for RPA.

[2] UD = Underivatizable

[3] MBC and 2-AB PFBC-derivatives give similar RRT.

Table 17. Retention times and response values relative to aldrin of PFBC derivatized pesticides (pyridine system) on 4% SE-30/6% OV-210 column, 205° C, (Att. = 4 x 10^{2}, N_2 flow = 80 ml/min Tracor 222, EC).

Pesticide	RRT	RPA
Amitrole	0.316	1.235
Avadex	UD ($\underline{4.404}$, 0.456, 0.670)[1]	(0.027)[2]
Azodrin	0.550	0.012
Benomyl[2]	14.300	0.077
Bux	0.825	0.004
Dicamba	UD	—
DNBP	UD	—
DNOC	UD	—
Carbofuran	0.825	0.001
IPC	UD	—
2,3,5-Landrin	0.705	0.008
3,4,5-Landrin	0.872	0.025
Monuron	2.980	0.730
Methomyl	UD	—
Phosphamidon	UD	—
Temik	UD	—
Zectran	1.020	0.001

[1] Data represents original structure of avadex; major peak (underlining) was computed for RPA.

[2] MBC and 2-AB PFBC-derivatives gave similar RRT.

Table 18. Retention times and response values relative to aldrin of PFBC derivatized pesticides (pyridine system) on 1.5% OV-17/1.95% OV-210 column, 205° C (Att. 4 x 10², N_2 flow = 60 ml/min. Tracor 222, EC).

Pesticide	RRT	RPA
Amitrole	0.522	0.072
Avadex	UD (0.24, 0.37, 0.74)[1]	(0.041)[1]
Azodrin	—	—
Benomyl[2]	14.00	0.196
Bux	0.784	0.001
Dicamba	UD	—
DNBP	UD	—
DNOC	UD	—
Carbofuran	0.74	0.155
IPC	0.61	0.001
2,3,5-Landrin	0.525	0.010
3,4,5-Landrin	0.670	0.050
Monuron	2.65	0.480
Methomyl	UD	—
Phosphamidon	UD	—
Temik	UD	—
Zectran	0.822	0.014

---

[1] Data represents original structure of avadex; only major peak (underlining) was computed for RPA.

[2] MBC and 2-AB PFBC-derivatives gave similar RRT.

Table 19. Retention times and response values relative to aldrin of PFBC derivatized pesticides (0.1 N NaOH system) on 1.5% OV-17/1.95% OV-210 column, 205° C (Att. = 4 x 10^2, N_2 flow = 80 ml/min. Tracor 222, EC).

Pesticide	RRT	RPA
Amitrole	UD	—
Avadex	UD	—
Azodrin	UD	—
Benomyl	UD	—
Bux	UD	—
Dicamba	UD	—
DNBP	UD	—
DNOC	UD	—
Carbofuran	UD	—
IPC	0.782	0.017
2,3,5-Landrin	0.580	0.540
3,4,5-Landrin	0.700	0.445
Monuron	1.57, 2.33	0.001, 0.005
Methomyl	UD	—
Phosphamidon	UD	—
Temik	UD	—
Zectran	13.25	0.055

Table 20. Retention times and response values relative to aldrin of PFBC derivatized pesticides (0.1 M NaOH system) on 4% SE-30/6% OV-210 column, 205° C (Att. = 4 x 10^2, N_2 flow = 50 ml/min. Tracor 222, EC).

Pesticide	RRT	RPA
Amitrole	UD	—
Avadex	UD	—
Azodrin	UD	—
Benomyl	UD	—
Bux	UD	—
Dicamba	UD	—
DNBP	UD	—
DNOC	UD	—
Carbofuran	UD	—
IPC	0.670	0.016
2,3,5-Landrin	0.710	0.212
3,4,5-Landrin	0.840	0.181
Monuron	1.70, 3.00	0.001, 0.004
Methomyl	UD	—
Phosphamidon	UD	—
Temik	UD	—
Zectran	19.4	0.031

In the 0.1 M NaOH system, no responses were obtained from amitrole, benomyl and most other pesticides (Table 18). Two low response GLC peaks were obtained from monuron which might be the result of its thermal degradation. Moderate sensitivities (RPAs 0.2-0.4) were obtained for Landrin (2,3,5 & 3,4,5) (Tables 19-20).

Retention times and response values relative to aldrin of α-bromo-2,3,4,5,6-pentafluorotoluene (BPFT) derivatized pesticides on two columns are presented in Table 21. The best result was obtained for dicamba (RPAs = 0.7-0.9). DNBP and monuron gave moderate sensitivities (RPAs = 0.13-0.20) and unacceptably low sensitivities were obtained from other BPFT derivatized pesticides (RPAs = 0.03-0.09).

Derivatization of DNOC, DNBP, and dicamba was attempted successfully by using diazomethane (Table 22). The relatively simple derivatization procedure (18) and clean reagent blank make it the most promising technique for the above pesticides. In addition, methylated derivatives of these compounds gave relatively high sensitivities, linear analytical curves, and good separation of GLC peaks (Figure 19 and Table 22).

Retention times and responses relative to aldrin of selected derivatization techniques for twelve multi-class pesticides are compiled from previous results in Table 23. Among the three selected derivatization techniques (PFPA, DAM and PFBC), PFPA was found to be the most suitable derivatization reagent for most of the tested carbamate pesticides (bux, carbofuran, IPC, Landrin-2,3,5, Landrin-3,4,5 and Zectran).

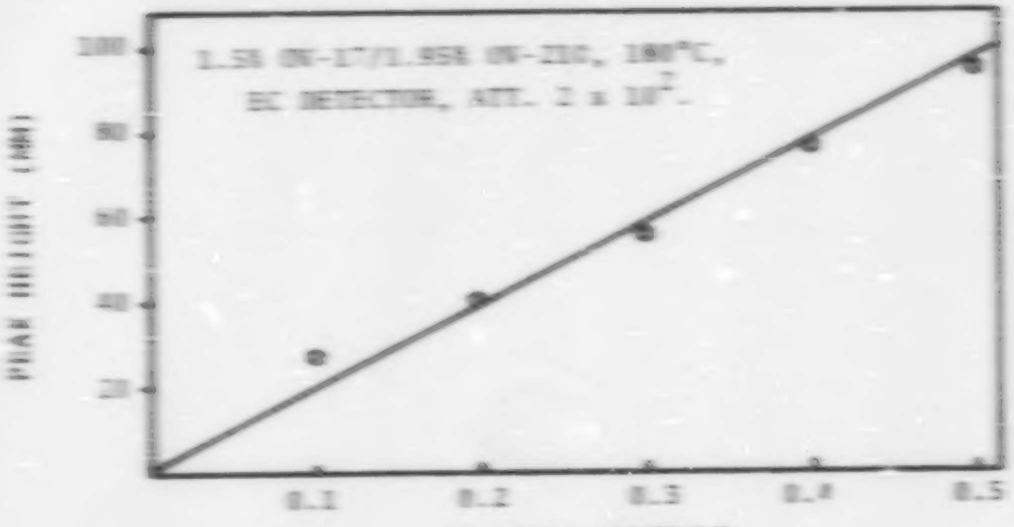


FIGURE 16

Analytical curve of PFBC-Amitrole

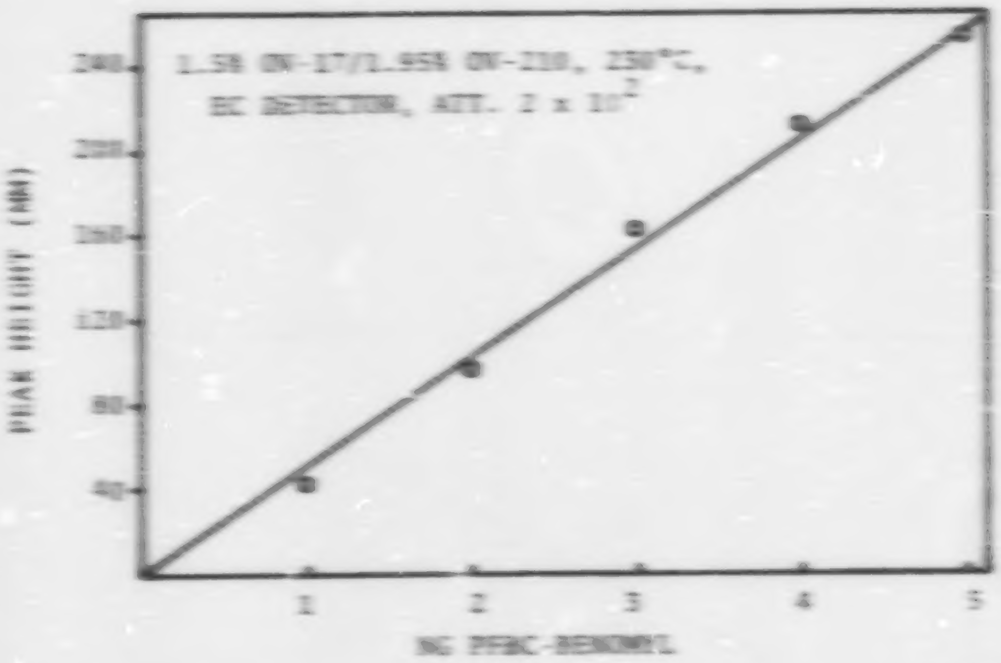


FIGURE 17

Analytical curve of PFBC-Benomyl

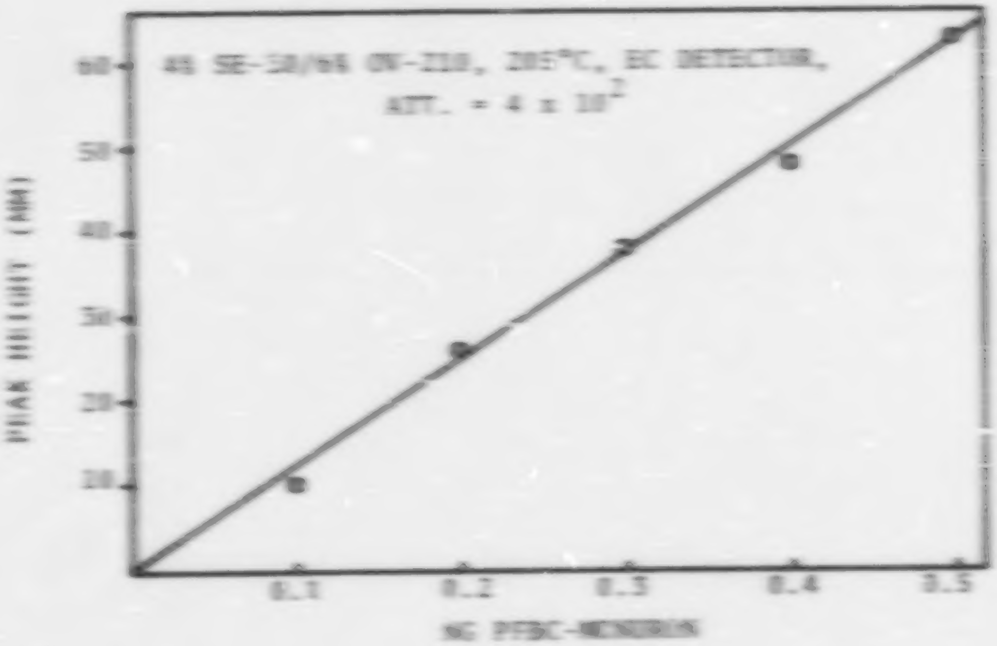


FIGURE 18

Analytical curve of PFBC-Monuron

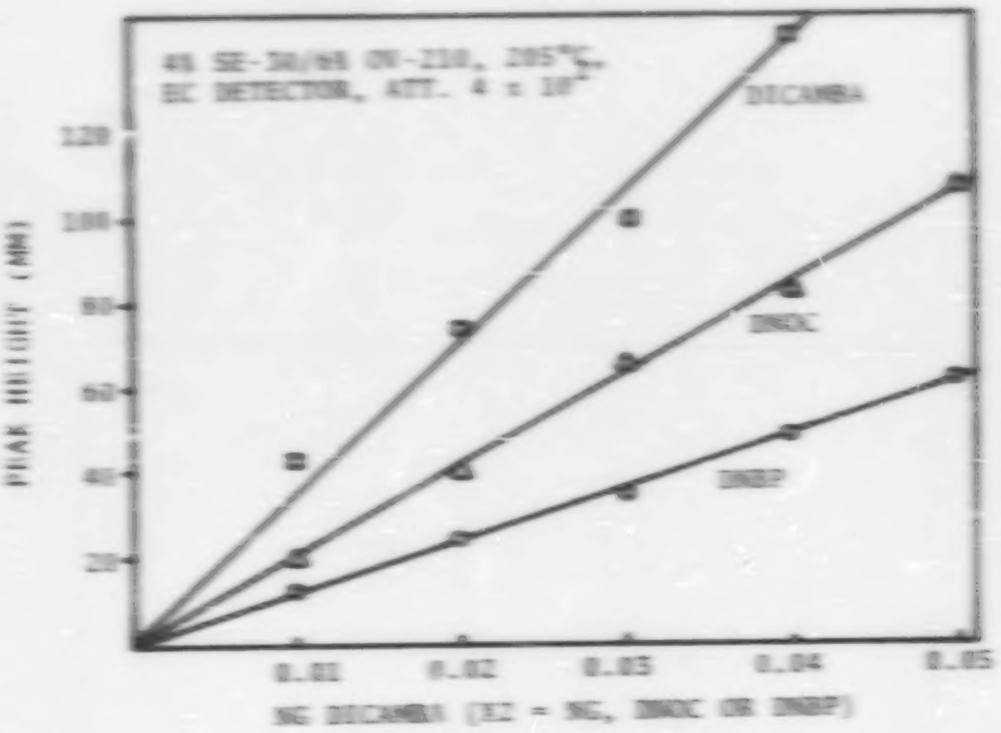


FIGURE 19

Analytical curves of Dicamba, DNOC and DNBP (Diazomethane Derivatives)

Table 21. Retention times and response values relative to aldrin of BPPI derivatized pesticides on two columns (Att. = 4 x 10^3, Tracor 222, column temp. 195°C, EC, pH 12 buffer system).

Pesticide	4% SE-30/6% OV-210 N_2 flow = 67 ml/min		1.5% OV-17/1.95% OV-210 N_2 flow = 53 ml/min	
	RRT	RPA	RRT	RPA
Azodrin	2.800	0.028	2.100	0.041
Dicamba	1.560	0.770	1.540	0.950
DNBP	6.175	0.150	4.730	0.156
DNOC	5.350	0.059	4.500	0.110
3,4,5-Landrin	3.075	0.069	2.770	0.098
Monuron	0.740	0.126	0.690	0.192
Methomyl	4.025	0.086	2.830	0.098
Temik	4.025	0.053	2.770	0.098

Table 21. Retention times and response values relative to aldrin of Diazomethane-derivatized pesticides on 4% SE-30/6% OV-210 column, 205°C (Att. = 4 x 10^2, N_2 flow = 67 ml/min, Tracor 222, EC).

Pesticide	RRT[1]	RPA[2]
DNOC	0.900	0.250
DNBP	1.300	0.160
Dicamba	0.300	0.610

[1] & [2] See footnotes of Table 2.

Note: Diazomethane-derivatized pesticides which produced no GC responses were 2,3,5-Landrin, carbofuran, Zectran, and Bux.

Table 23. Retention times and response values relative to aldrin of selected derivatization techniques on twelve multi-class pesticides.

Pesticide	RRT[1]	RPA[2]	Analytical Curve[3] (Figure No.)
PFPA Derivatization (Ref. 1)[4]			
IPC	0.142	0.286	15
Landrin-2,3,5	0.321	0.100	16
Landrin-3,4,5	0.420	0.290	16
Bux	0.43, 0.54	0.142, 0.047	16
Carbofuran	0.462	0.148	16
Zectran	0.536	0.105	16
DAM Derivatization (Ref. 3)[4]			
Dicamba	0.300	0.368	14
DNOC	0.900	0.230	14
DNBP	1.300	0.190	14
PFBC Derivatization, pyridine system (Ref. 5)[4]			
Amitrole	0.522	0.072	17
Monuron	2.980	0.730	19
Benomyl	12.500	0.033	18

[1&2] See footnotes of Table 2.

[3] See Figures for GLC operating conditons.

[4] PFPA = Pentafluoropropionic anhydride; DAM = Diazomethane; PFBC = Pentafluorobenzoyl chloride.

PFPA-derivatives of the above carbamates gave relatively high sensitivities with the EC detector (RPA between 0.10 and 0.29, Table 23) and also demonstrated a high degree of reproducibility along with relatively linear analytical curves (Figures 14 and 15). A disadvantage of PFPA-derivatized pesticides over other methods (DAM and PFBC) was their relatively short retention times (RRT = 0.14-0.53, Table 23), and they were eluted from GLC columns too close together, although they were slightly different in RRT and could ultimately be differentiated from each other. Problems were encountered when these carbamate pesticides were studied for low level (0.1 and 0.01 ppm) soil recovery; this will be discussed in detail later.

Negative derivatization results were obtained with the following reagents: 9-chloromethylanthracene (CMA), acetic acid N-hydroxy succinimide ester (AAHSE), N-metnyl-bis-(heptufluorobutyryl) amide (MHFBA), and N-methyl-bis-(trifluoroacetamide) (MBTFA).

GLC Detection of Sulfur-Containing Pesticides

None of the tested reagents produced suitable derivatives of Temik or methomyl for either the sulfur mode of the FPD or the EC detector. However, the non-specific methylamine derivatization of Temik and methomyl by DBSC was possible as previously mentioned. Figure 20 presents analytical curves of Temik and methomyl which were obtained by the utilization of the multi-residue, 4% SE-30/6% OV-210 (150°C) column and detected by the sulfur mode of the FPD. Lack of sensitivity on the above column led to the selection of a second rather polar column (24), 5% Carbowax 20 M, (Figure 21), on which sensitivities of Temik and methomyl could be increased two and fifteen fold respectively. The analytical scheme was based on the thermal degradation of Temik or methomyl to nitrile derivatives at an injection port temperature of 300°C (24, 26). Sulfur dioxide added to the hydrogen gas of the FPD increased the sensitivity of Temik and methomyl by a factor of 5 (Figure 21). Zehner and Simonaitis (25) claimed an 8-fold increase in the sensitivity of Temik and malathion when SO_2 was introduced.

Loss of Pesticides in the Concentration Steps (Kuderna-Danish concentrator or evaporation of solvents under room temperature in hood)

The stability and recovery of nitrogen-containing pesticides in the Kuderna-Danish concentrator step were briefly investigated (Table 24) before any soil residue studies were undertaken. Only captan and methomyl were lost in unacceptable amounts (73 and 84% respectively) in the Kuderna-Danish concentration step. The interaction between methanol and captan under elevated temperature was believed to be the major factor contributing to the disappearance of captan. Surprisingly, methomyl could be recovered quantitatively after it was heated at 95°C in 1:1 benzene:methanol for 30 min (see footnote of Table 24) in a sealed test tube.

Paraffin oil acted as a useful residue keeper as demonstrated in Table 25. Thirty-five and 22 percent of Avadex could be kept by 1 ml of 1% paraffin oil in benzene when the dry residue was subjected to continuous evaporation under a hood for 15 min and 16 hr respectively. Fifty percent of Zectran could

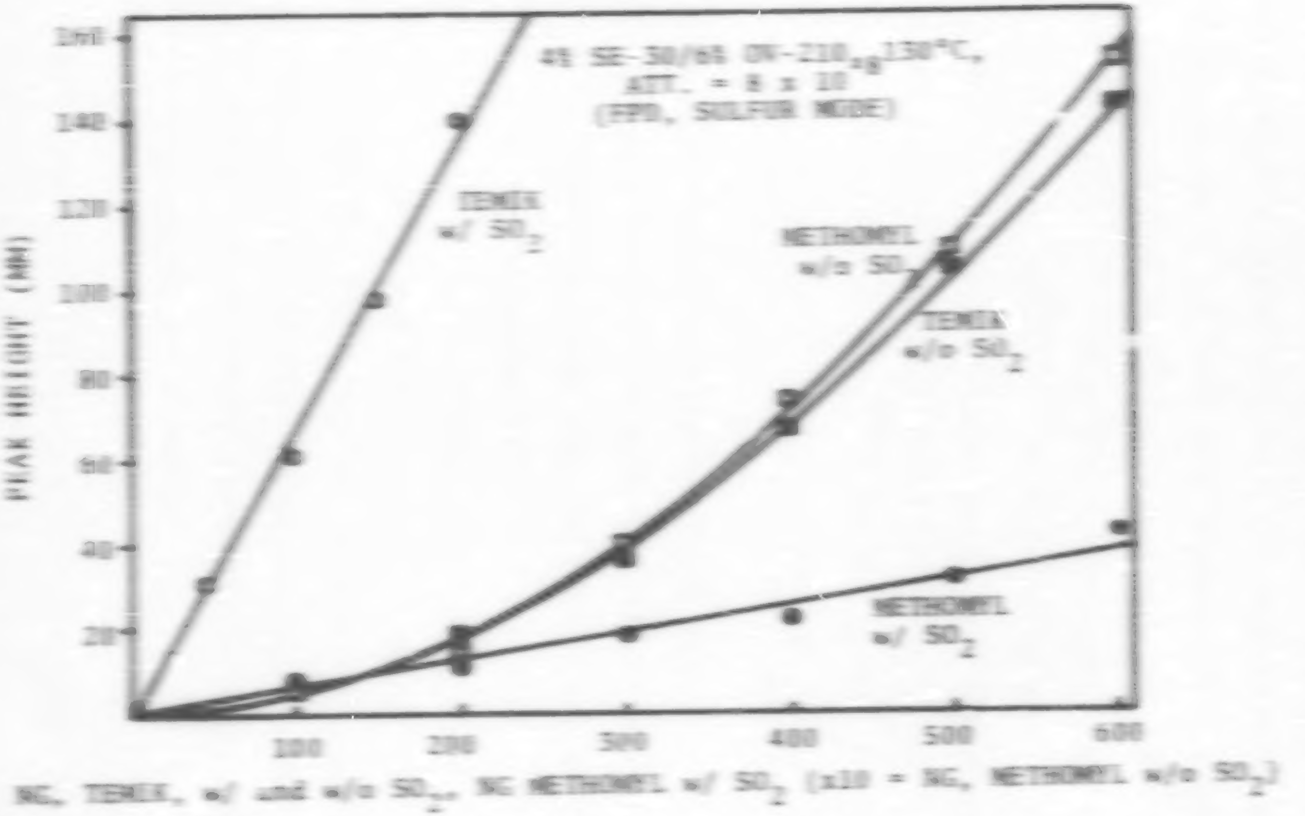


FIGURE 20

Analytical curves of Temik and Methomyl.

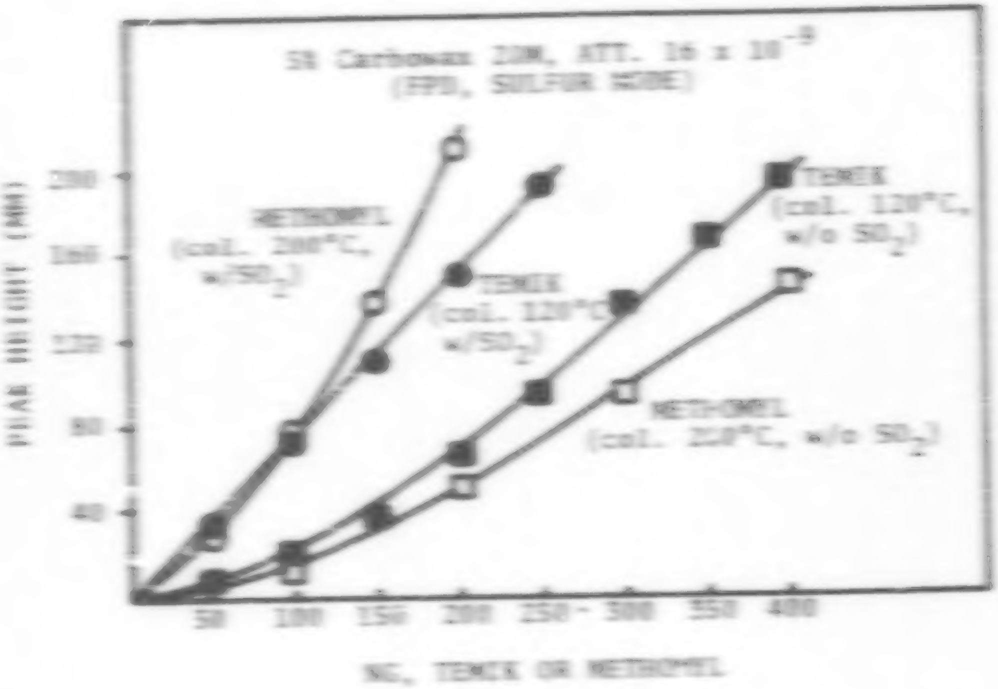


FIGURE 21

Analytical curves of Temik and Methomyl.

be preserved by paraffin oil even when its dry residue was subjected to continued evaporation under a hood for 48 hr.

Table 26 and Figure 22 present the degradation of seven carbamate and substituted urea pesticides in benzene/methanol (1:1) for 6 hr at elevated temperature (100°). The loss ranged from 15% for Landrin - 3,4,5 to 83% for carbofuran.

Silica gel column chromatography

Pesticide standards were studied for recoveries and elution patterns from a silica gel column prior to soil recovery studies (Figures 23 a and 23 b). Halogenated and organophosphate pesticides outlined in Figure 23 a were recovered quantitatively (79-105%) in a single fraction. Recoveries of 79 to 90% were obtained for endosulfan, Avadex, Dursban, phorate, methyl parathion, parathion, CIPC, diazinon, demeton, dimethoate, phosphamidon, and Azodrin. PCNB, trifluralin, Perthane, methoxychlor, captan, folpet, Difolatan, chlorobenzilate, malathion, azinphos methyl, azinphos ethyl, atrazine, simazine and bromacil were recovered at 90 to 105%.

Elution patterns of multi-class pesticides are outlined in Figure 23 b. Pesticides which could be recovered from the column quantitatively (over 75%) in a single fraction were DNBP, benomyl, DNOC, bux, IPC, Zectran, carbofuran, monuron, and amitrole. Pesticides which could be recovered quantitatively (over 75%) from more than one elution fraction were 2,3,5-Landrin (46% fraction III; 58% fraction IV), and 3,4,5-Landrin (62% fraction III; 20% fraction IV). The dicamba standard was lost on the silica gel column; however, DAM dicamba could be eluted

Table 24. Percent recovery of 500 ug of pesticides each in the Kuderna-Danish concentrator step concentrated from 250 ml 1:1 benzene/methanol to 10 ml.

Pesticide	Detection Techniques	% Recovery
Avadex[1]	EC, non-derivatized	85
Captan[1]	EC, non-derivatized	27
Bux	EC, PFPA[4]	95
Carbofuran	EC, PFPA[4]	97
Zectran	EC, PFPA[4]	93
Monuron	EC, PFBC	96
Temik	FPD, S-mode[3]	103
Methomyl[2]	FPD, S-mode[3]	16

[1] Sample of avadex standard (5 g) in 1 ml of 1:1 benzene/methanol was heated at 95° C for 48 hrs in a sealed Teflon-capped test tube, 86% of the heated Avadex was recovered; while samples of captan, folpet and Difolatan underwent the same treatment, no recoverable residues were observed.

[2] Methomyl was recovered quantitatively when it was heated at 95°C for 30 min in a sealed test tube in 1:1 benzene/methanol.

[3] See Figure 21 for GLC operating conditions.

[4] Samples of pesticides should be free from methanol before PFPA derivatization, since methanol inhibited PFPA-pesticide reaction.

Table 25. Effect of paraffin oil keeper on pesticide retention.

Pesticide (5 µg)	Time after complete dryness of residue	% pesticide recovered	
		without paraffin	with paraffin[2]
Avadex	15 min	65	100
Avadex	16 hr	22	44
Zectran	48 hr	50	100

[1] Avadex was selected as a model pesticide because it is the most volatile pesticide under investigation.

[2] One ml of 1% paraffin oil in benzene was added to each sample. No interfering GLC peaks for paraffin oil were observed.

Table 26. Recovery of 100 µg of nitrogen containing pesticides after heating for 6 hr (100°C) in a capped test tube with 2 ml benzene/methanol (1:1).

Pesticide	RRT	% recovery[2]
IPC	0.18	74.0
Landrin, 2,3,5	0.35	70.1
Bux	0.40, 0.43	28.0
Carbofuran	0.41	17.3
Landrin, 3,4,5	0.42	85.0
Zectran	0.51	51.5
Monuron	0.55	48.0

[1] GLC conditions: 4% SE-30/6% OV-210, 205°C, Att. = 2 x 10^2, Tracor 222, EC.

[2] Average of duplicate samples.

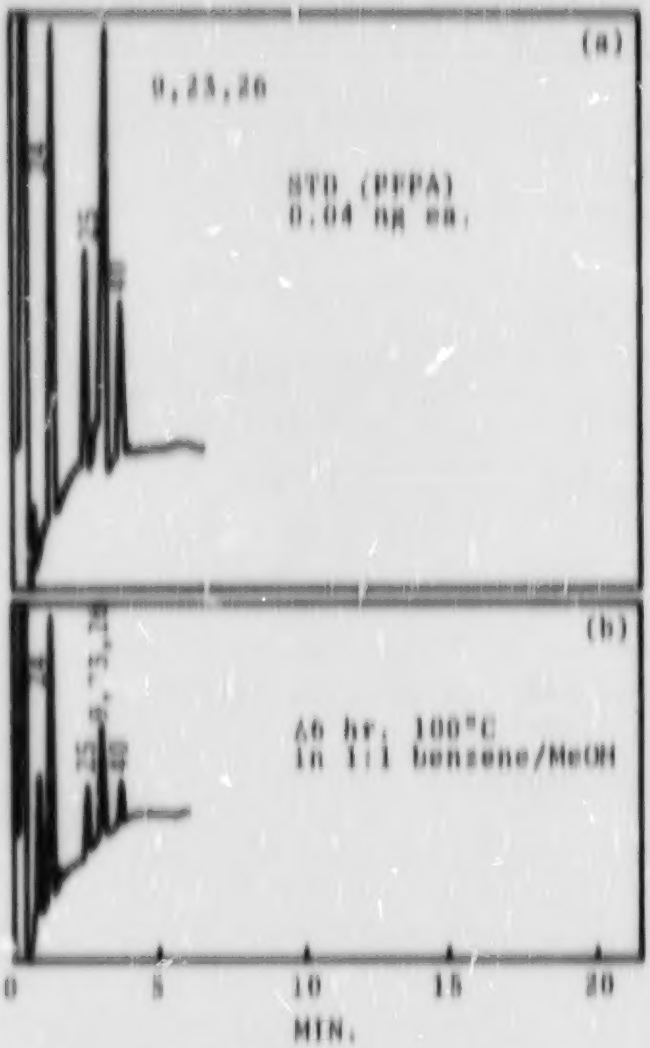


FIGURE 22

Chromatograms of standard PFPA derivatives of six carbamate pesticides (a) and after they were heated at 100°C in a sealed test tube for 6 hr in 1:1 benzene/MeOH (b). Conditions as in Table 26.

quantitatively (85%) in fraction II. PFBC-benomyl could be recovered in fraction II (80%) and fraction III (15%), and PFBC-amitrole was eluted in fraction IV (50%) and fraction V (48%). Interference due to the PFBC reagent were removed by the silica gel column. The major percentage (~70%) of the unreacted PFBC reagent was observed in fraction I and some (~25%) was carried over to fraction II. All residue keeper (paraffin oil) was eluted in fraction I from the silica gel column. Fairly good yield (84%) of methomyl was observed while a lower yield (66%) was obtained for Temik.

Co-extractive interferences from crude soil extracts and clean-up by silica gel column chromatography.

Most gas chromatograms were derived from the multiple recoveries of those forty pesticides (Table 1) in a single sandy soil sample (0.01 ppm) by Soxhlet extraction. Relatively low sensitivity pesticides which could not be presented in the same chromatogram because of interferences from other more sensitive pesticides are illustrated by broken line peaks.

1. Halogenated pesticides (EC). Chromatograms for a crude control sandy soil extract and soil spiked with fourteen EC-sensitive pesticides in five silica gel fractions and their respective controls are presented in Figures 24a-24f.

Figure 24a presents the background interference of crude benzene/methanol sandy soil extract for 10 mg soil equivalent. Complete recovery of 0.01 ppm PCNB from sandy soil is seen in Figure 24b (Fraction I, Hexane). In fraction II, Avadex, Trifluralin, methoxychlor, and endosulfan were quantitatively

Figure 25a. Outline of Halogenated and Organophosphate Pesticide Standards (1 mg each) eluted from the silica gel column (figures immediately after pesticide indicate percent recoveries).

Fraction I Hexane

PCNB (94%)

Fraction II 60% Benzene/Hexane

trifluralin (105%)
Perthane (97%)
methoxychlor (92%)
endosulfan (90%)
Avadex (79%)
Durshan (85%)
phorate (79%)
methyl parathion (88%)
parathion (87%)

Fraction III 5% Acetonitrile/Benzene

CIPC (90%)
captan (102%)
folpet (98.5%)
Difolatan (100%)
chlorobenzilate (91%)
diazinon (87%)
malathion (95%)
azinphos methyl (100%)
azinphos ethyl (94%)

Silica gel
(16 g)

Na_2SO_4
(14g)

Fraction IV 10% Acetonitrile/Benzene

atrazine (100%)
simazine (100%)
demeton (80%)

Fraction V 20% Acetonitrile/Benzene

bromacil (98%)
phosphamidon (79%)
dimethoate (81%)

Fraction VI Acetonitrile

Azodrin (90%)

Figure 23b. Outline of Multiclass pesticide standard (1 mg) eluted from silica gel column (figures immediately after pesticide indicate percent recoveries).[1]

Fraction I Hexane

No pesticides (paraffin oil)

Fraction II 60% Benzene/Hexane

DNBP (83%)

DAM-dicamba (85%)

PFBC-benomyl (80%)

Fraction III 5% Acetonitrile/Benzene

benomyl (75%)

DNOC (83%)

2,3,4-Landrin (46%)

3,4,5-Landrin (62%)

PFBC-benomyl (15%)

bux (95%)

IPC (93%)

Zectran (90%)

Silica gel (16 g)

Na_2SO_4 (14 g)

Fraction IV 10% Acetonitrile/Benzene

2,3,5-Landrin (38%)

3,4,5-Landrin (20%)

Temik (66%)

Carbofuran (88%)

PFBC-amitrole (50%)

Fraction V 20% Acetonitrile/Benzene

methomyl (84%)

monuron (95%)

PFBC-amitrole (48%)

Fraction VI Acetonitrile

amitrole (100%)

Unrecoverable

dicamba

---

[1] Some pesticides were eluted in more than one fraction (underlined); addition of those fractions represents total recoveries; recovery values were estimated by single analysis of pesticide standards without addition of soil.

recovered from the sandy soil sample (91-110%) (Figure 24c). The chromatogram for Perthane was obtained from the recovery study of the individual compound because endosulfan, when in the same soil sample, masked the less sensitive Perthane peak.

In fraction III captan, folpet, and Difolatan were recovered quantitatively (76-89%) using the Polytron extractor with benzene/methanol (1:1) as the extracting solvent (Figure 24d). CIPC, fortified at 0.10 ppm, was partially obscured by one sandy soil coextractive peak which prevented accurate estimation. Complete recovery (100%) was obtained for chlorobenzilate added to a sandy soil sample which evidenced a minor interference peak. (Figure 24d).

Simazine and atrazine were both recovered in fraction IV (Figure 24e). No GLC column under investigation could be used to separate these two compounds, although they could be recovered from soil quantitatively (83-97%). High recovery (95%) was obtained for bromacil in fraction V (Figure 24f).

2. Organophosphate pesticides. No interference peaks from the column blank, clean-up or crude control soil samples (500 mg soil equivalent) were observed with the FPD (Figure 25a). In fraction II (Figure 25a), recovery results were fair (66-77%) for Dursban, methyl parathion, and parathion and poor (57%) for phorate. In fractions III-VI, shown in Figures 25a and 25b, poor recovery results (40-41%) were obtained for diazinon and demeton. Good recoveries (76-100%) were obtained for malathion, azinphos ethyl, and azinphos methyl (Fraction III); dimethoate and phosphamidon (Fraction V); and Azodrin (Fraction VI). Gas

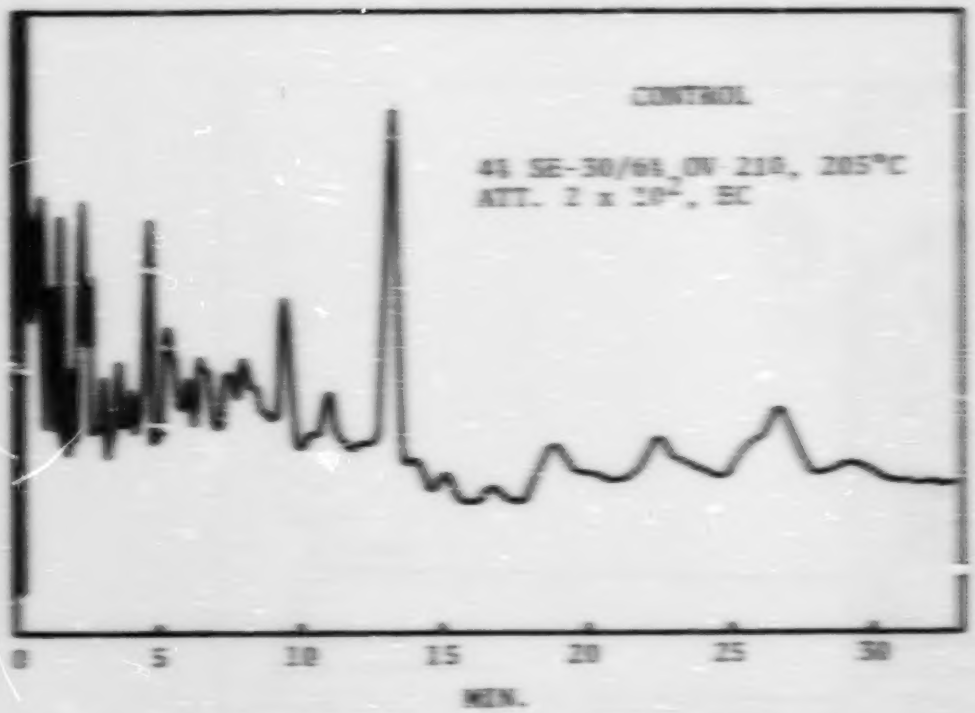


FIGURE 24 a

Gas chromatogram of crude control sandy soil extract (10 mg soil equivalent).

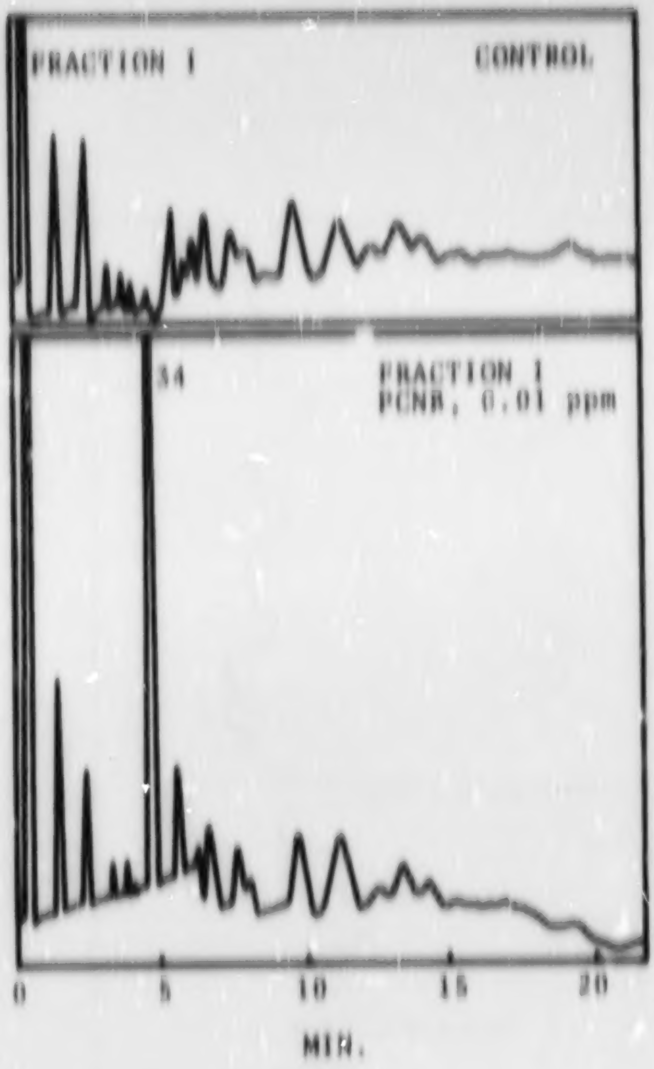


FIGURE 24 b

Gas chromatograms of control and 0.01 ppm PCNB from sandy soil (10 mg soil equivalent). G.C conditions as for Fig. 24 a.

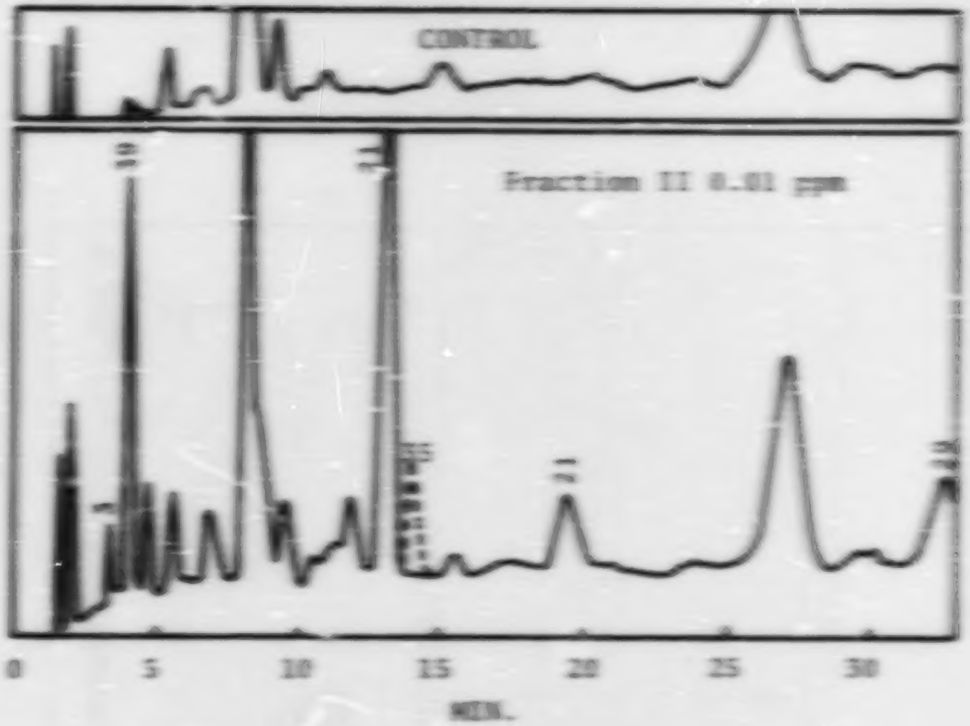


FIGURE 24 c

Gas chromatograms of 0.01 ppm in sandy soil of Avadex (3), trifluralin (39), endosulfan (21), methoxychlor (29), and Perthane (35) and the respective control (50 mg soil equivalent). G.C. conditions as for Fig. 24 b.

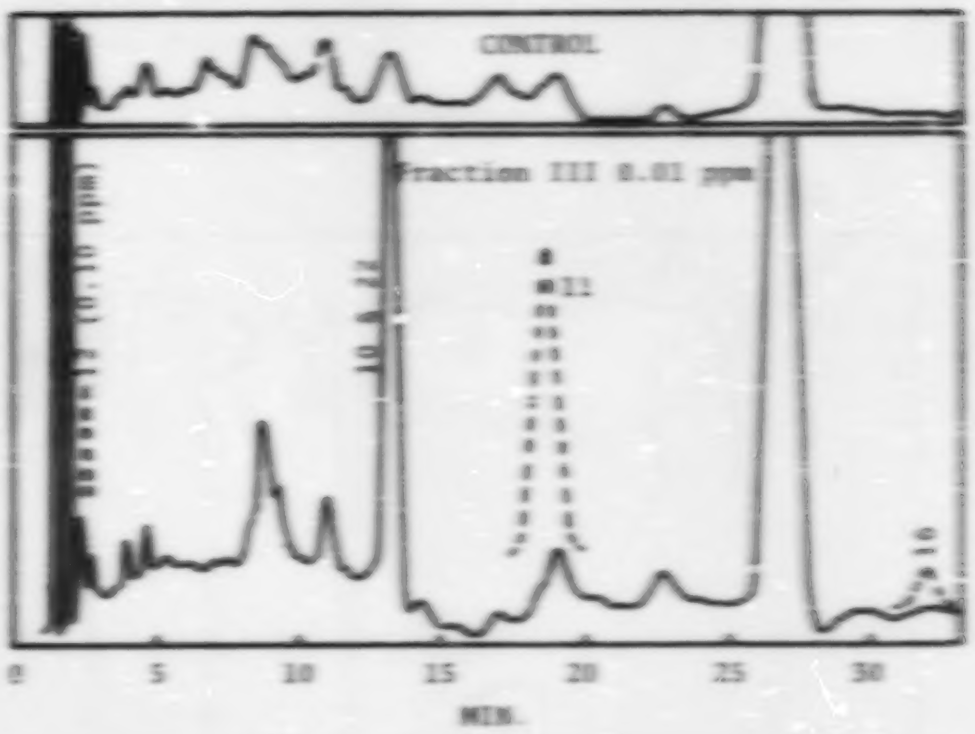


FIGURE 24 d

Gas chromatograms of 0.01 ppm in sandy soil of CIPC (12), captan (10), folpet (22), chlorobenzilate (11) and Difolatan (16) and the respective control (10 mg soil equivalent). G.C. conditions as for Fig. 24 a.

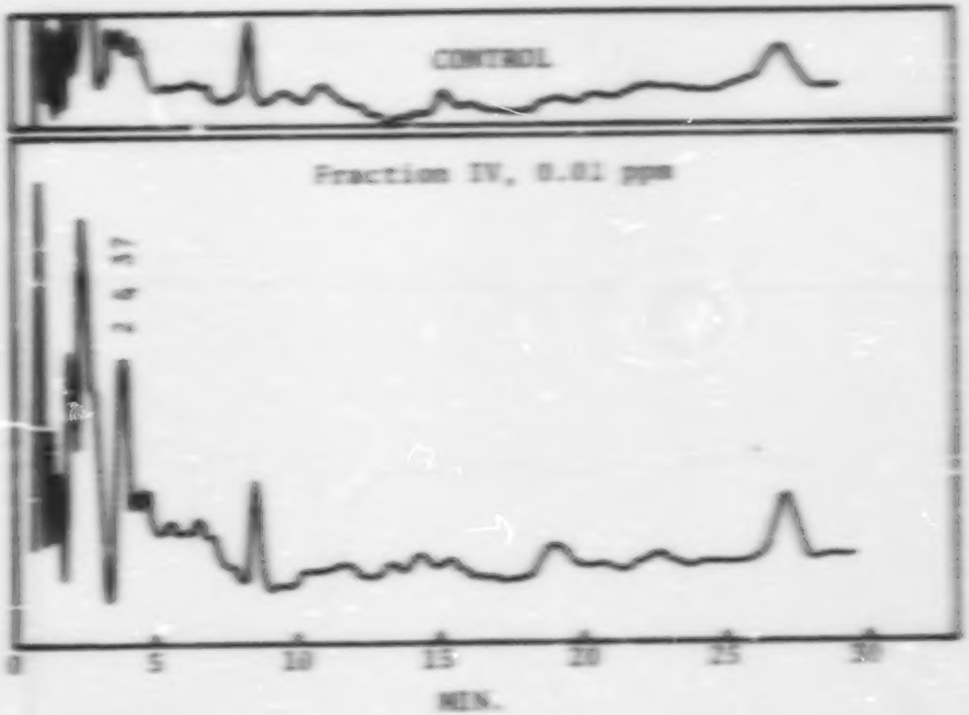


FIGURE 24 e

Gas Chromatograms of 0.01 ppm in sandy soil of atrazine (2) and simazine (37) and the respective control (50 mg soil equivalent). G.C. conditions as for Fig. 24 a.

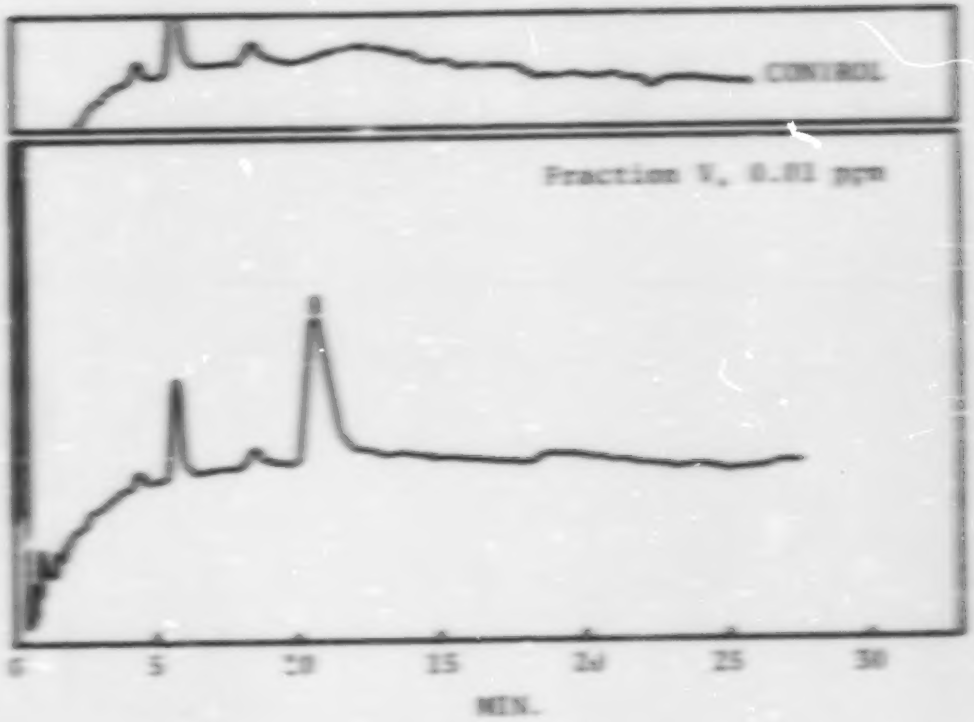


FIGURE 24 f

Gas chromatograms of 0.01 ppm in sandy soil of bromacil (8) and the respective control (50 mg soil equivalent). G.C. conditions same as for Fig. 24 a.

chromatograms of 23 OP pesticide standards are presented in Figures 25c and 25d.

3. Derivatization of pesticides. Chromatograms of DAM-control crude sandy soil sample (2 mg soil equivalent) and of 0.01 ppm of dicamba, DNOC and DNBP extracted from soil are presented in Figure 26. Recoveries were 59%, 73% and 103% respectively.

Figure 27 (a-d) presents the chromatograms of 0.1 or 1 ppm of six carbamate pesticides recovered from sandy soil samples by the Polytron extractor and clean up by a silica gel column. Figures 27a-27c present the chromatograms and recoveries of some carbamate pesticides eluted in fraction III (5% acetonitrile/benzene). Figure 27d represents the chromatograms for control soil and soil fortified with 0.1 ppm and eluted in fraction IV (20% acetonitrile/benzene). High background interferences were observed for all PFPA-derivatized carbamate pesticides.

Chromatograms of PFBC-derivatized monuron and benomyl recovered from sandy soil are presented in Figures 28a and 28b respectively. Monuron (0.01 ppm) was recovered in relatively low yield (50%) whereas benomyl could not be detected below the 0.1 ppm level due to coextractive interference (Figure 28b), although 90% recovery was obtained at the 0.1 ppm level.

Recoveries of Pesticides from Soils by Soxhlet Extraction

Soxhlet extraction for 12 hr exhibited superior efficiency to 1 hr tumbling for recovery of phorate and parathion from soil (Table 27). Therefore, most of the recovery studies were carried

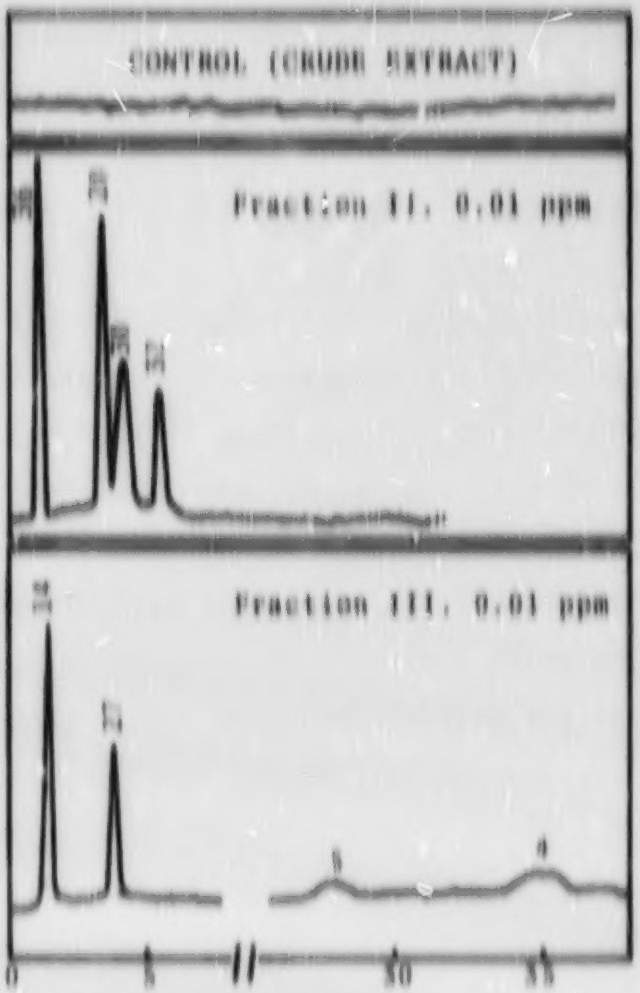


FIGURE 25 a

Top, control (500 mg soil equivalent), middle, chromatogram of 0.01 ppm OP pesticides in fraction II (60% B/H) and, bottom, fraction III (5% A/B). Peaks are for Phorate (36), Dursban (20), methyl parathion (30), parathion (32), diazinon (14), malathion (27), azinphos methyl (5), and azinphos ethyl (4). G.C. conditions as for Fig. 24 a.

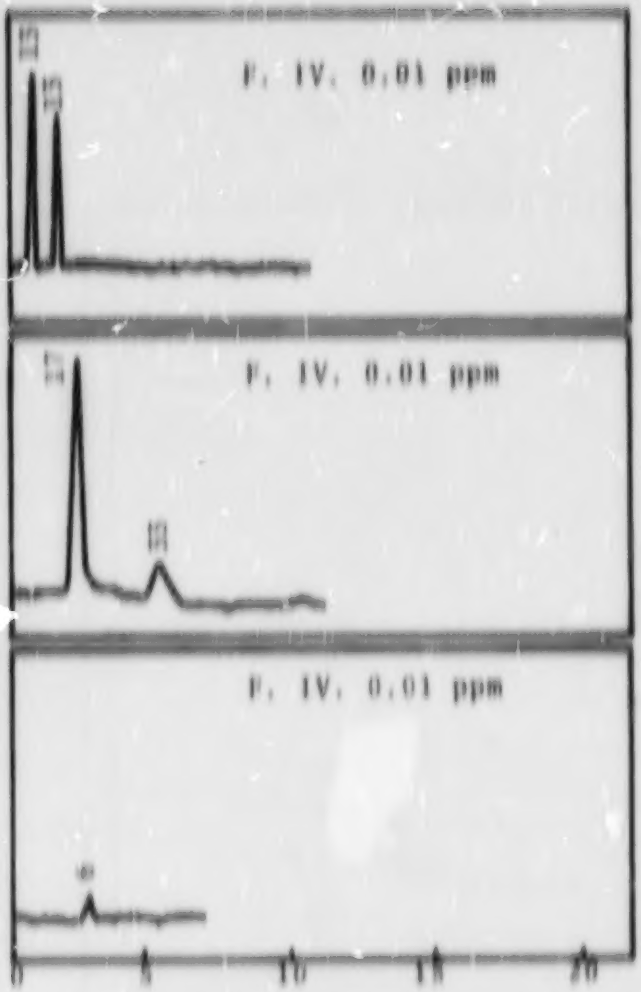


FIGURE 25 b

Chromatograms of 0.01 ppm OP pesticide in fraction IV (10% A/B), fraction V (20% A/B) and fraction VI (100% A/B). Peaks are demeton (13), dimethoate (17), phosphamidon (33), and Azodrin (6). G.C. conditions as in Fig. 24a.

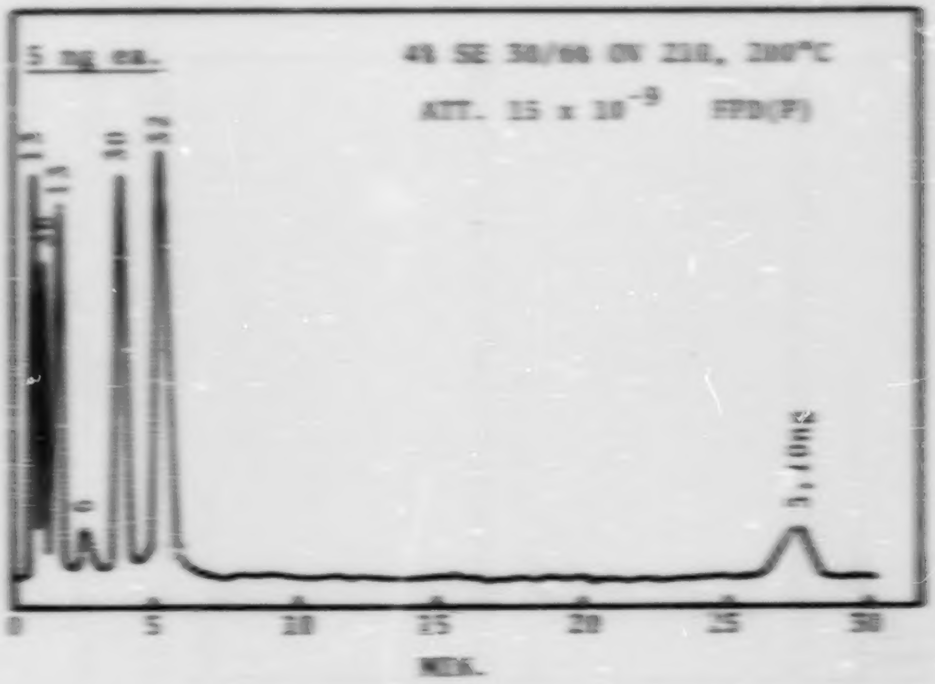


FIGURE 15 c

Gas chromatograms of 6 FPD (P)-sensitive pesticide standards. Peaks are demeton (13), phorate (36), Azodrin (6), methyl parathion (30), parathion (32), and azinphos ethyl (5).

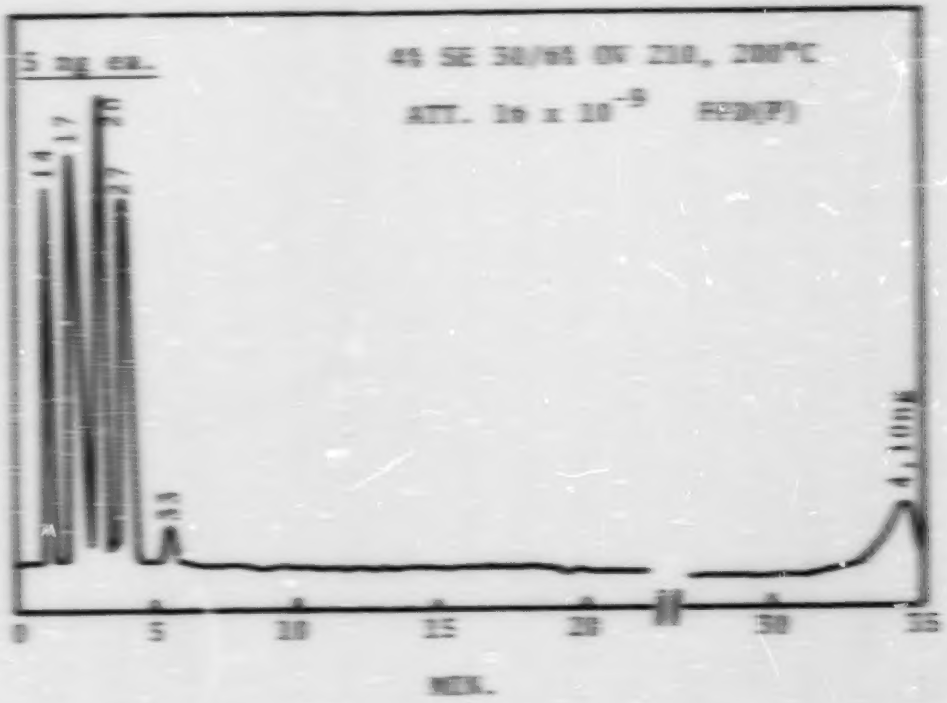


FIGURE 25 d

Gas chromatograms of 6 FPD (P)-sensitive pesticide standards. Peaks are diazinon (14), dimethoate (17), Dursban (20), malathion (27), phosphamidon (33), and azinphos ethyl (4).

out by the Soxhlet extraction method using 1:1 benzene/methanol as the extracting solvent. For those compounds unrecoverable by the above method, alternative techniques were used (Polytron), and their conditions were noted. Soil samples were screened, air-dried and characterized (Table 28). Air dried soils were adjusted to have 10% moisture content before the addition of pesticide standards for subsequent recovery studies.

1. Halogenated pesticides. Table 29 shows that recoveries were above 70% for all compounds in silty loam soil except bromacil (63% at 1 ppm), endosulfan (68% at 0.1 ppm) and trifluralin (50% at 0.01 ppm and 67% at 0.1 ppm).

Captan, folpet and Difolatan reacted with methanol during Soxhlet extraction; this phenomena was proven by simply refluxing these compounds in methanol for one hour. After this treatment, no captan, folpet or Difolatan peaks could be detected. When benzene-tumbling was substituted for Soxhlet extraction of these compounds, the recoveries were found to be 70 to 100%.

In sandy soil (Table 30), some low recovery results were obtained from soil samples for bromacil (61.3% at 0.1 ppm and 56% at 1 ppm), endosulfan (63% at 0.1 ppm and 1 ppm), and trifluralin (59.5, 50.0, 52.5% at 0.01, 0.10 and 1.0 ppm respectively). In sandy loam soil (Table 31), only trifluralin gave low recovery results for 0.01, 0.1 and 1 ppm (47-49%). The consistently low recovery of trifluralin from soils when the pesticide was present below 10 ppm might be an indication that trifluralin was tightly bound by soil particles. When the soil

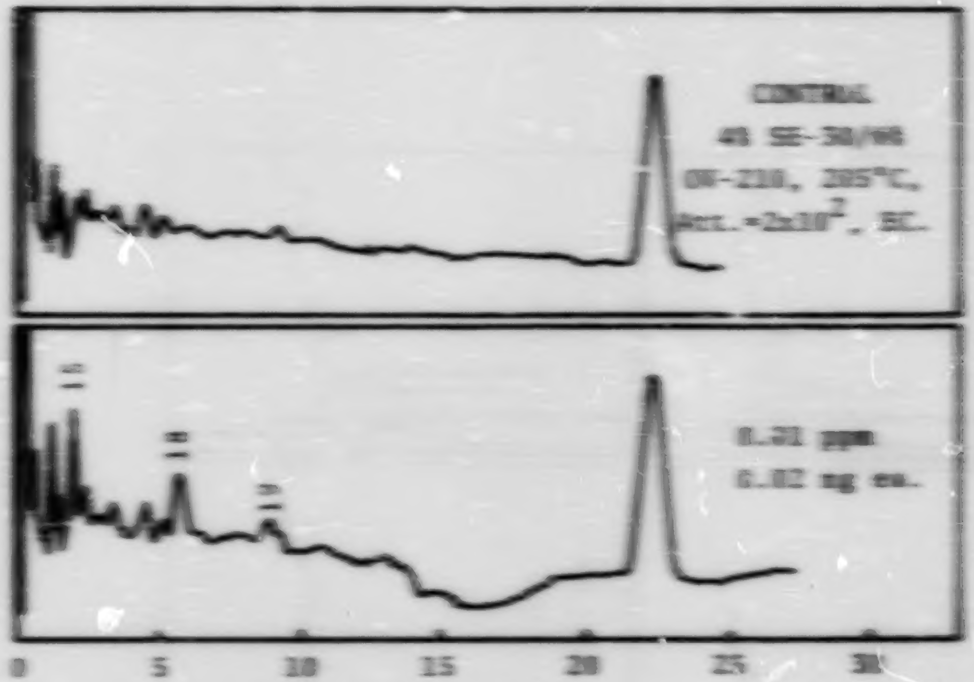


FIGURE 26

Chromatograms of control crude sandy soil (2 mg soil equivalent) and recoveries of 0.01 ppm of dicamba (13), DNOC (18), and DNBP (15) by diazomethane derivatization.

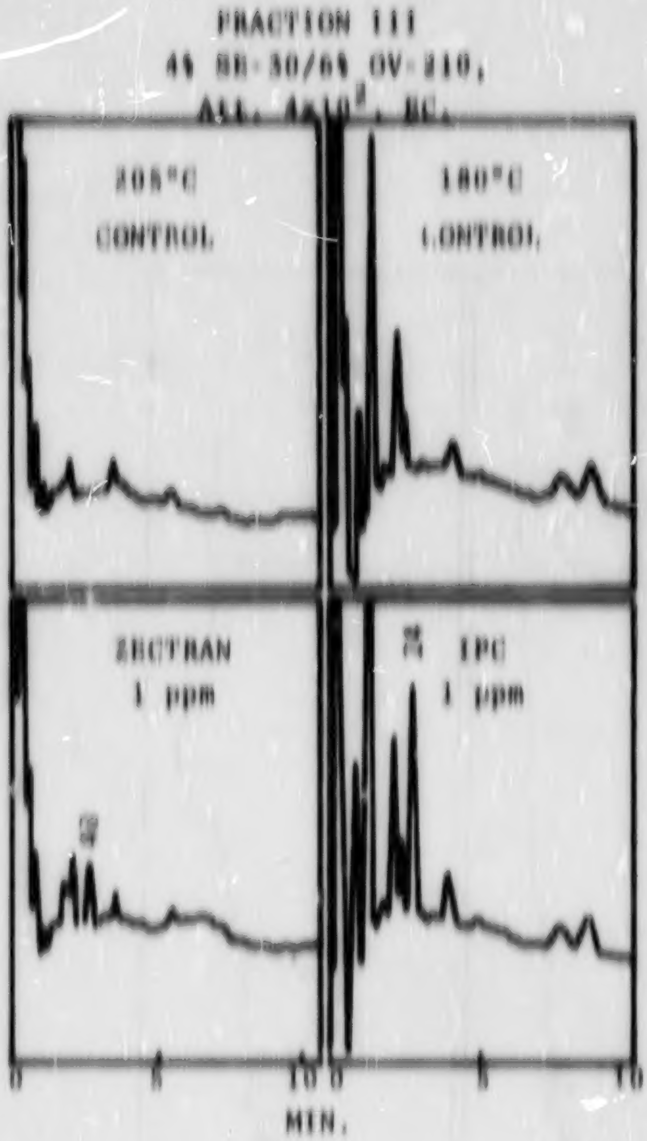


FIGURE 27 a

Chromatograms, left, of 1 ppm Zectran (40), and the respective control, right, 1 ppm IPC (24) and its control (1 mg soil equivalent), PFPA derivatives.

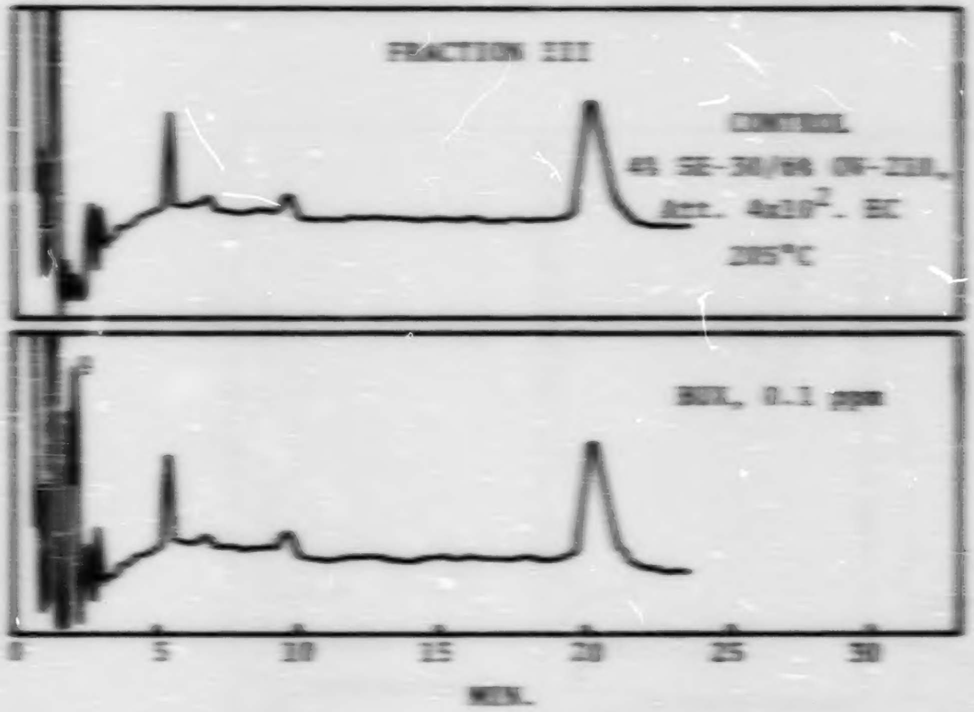


FIGURE 27 b

Chromatograms of 0.1 ppm bux (9) and its respective control (1 mg soil equivalent), PFPA derivative.

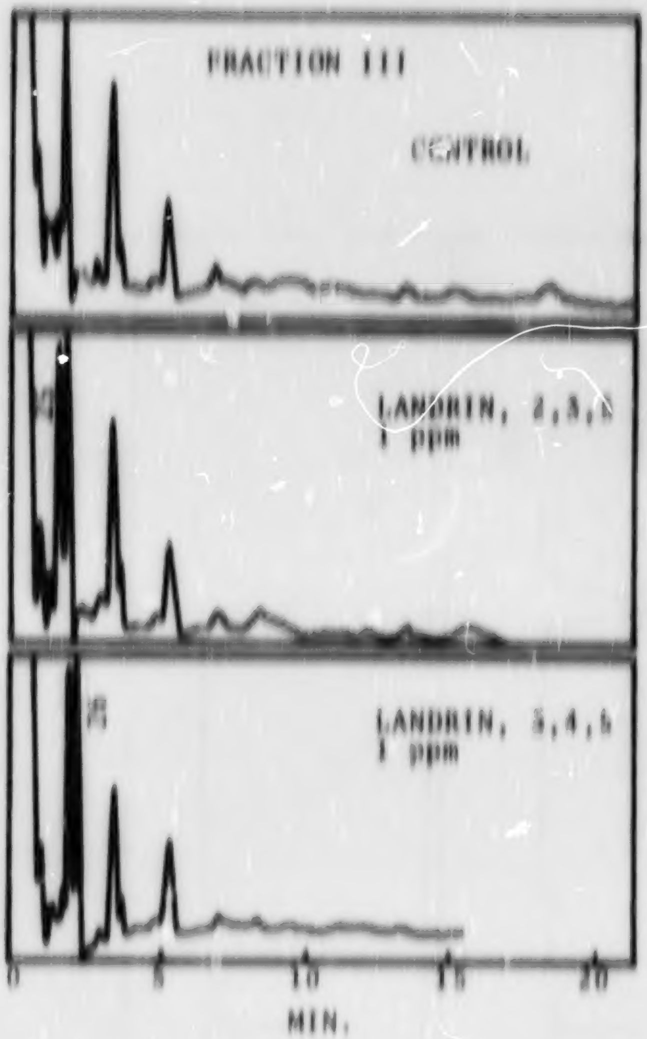


FIGURE 27 c

Gas chromatograms of Landrin (2,3,5 and 3,4,5 isomers) (25, 26) and the respective control (1 mg soil equivalent), PFPA derivatives. G.C. conditions as in Fig. 27 b.

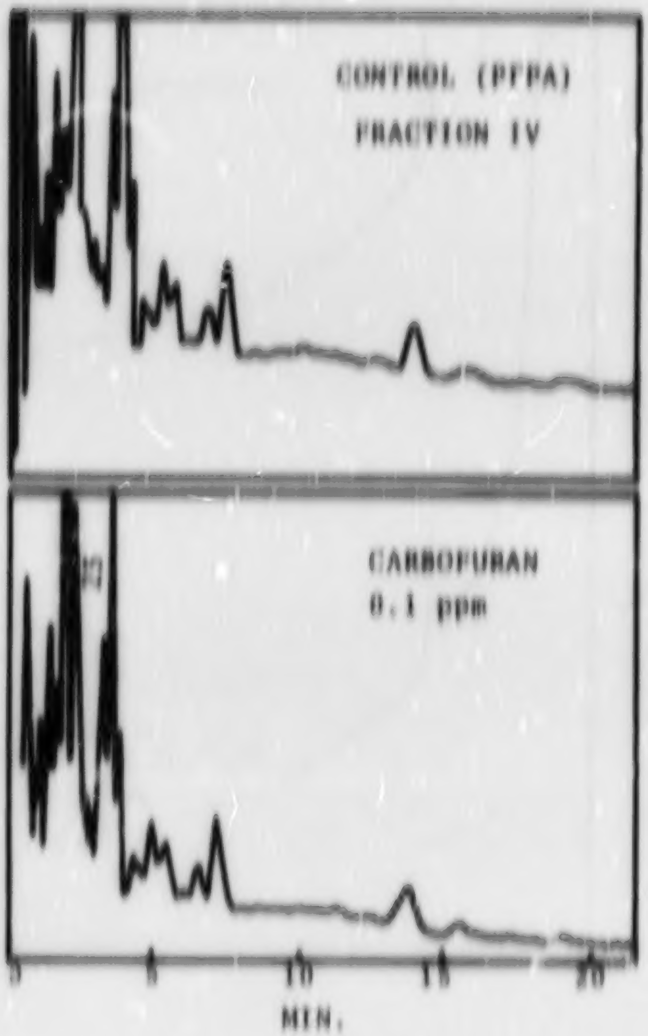


FIGURE 27 d

Chromatograms of 0.1 ppm carbofuran and its control (1 mg soil equivalent), PFPA derivative. G.C. conditions as in Fig. 27 b.

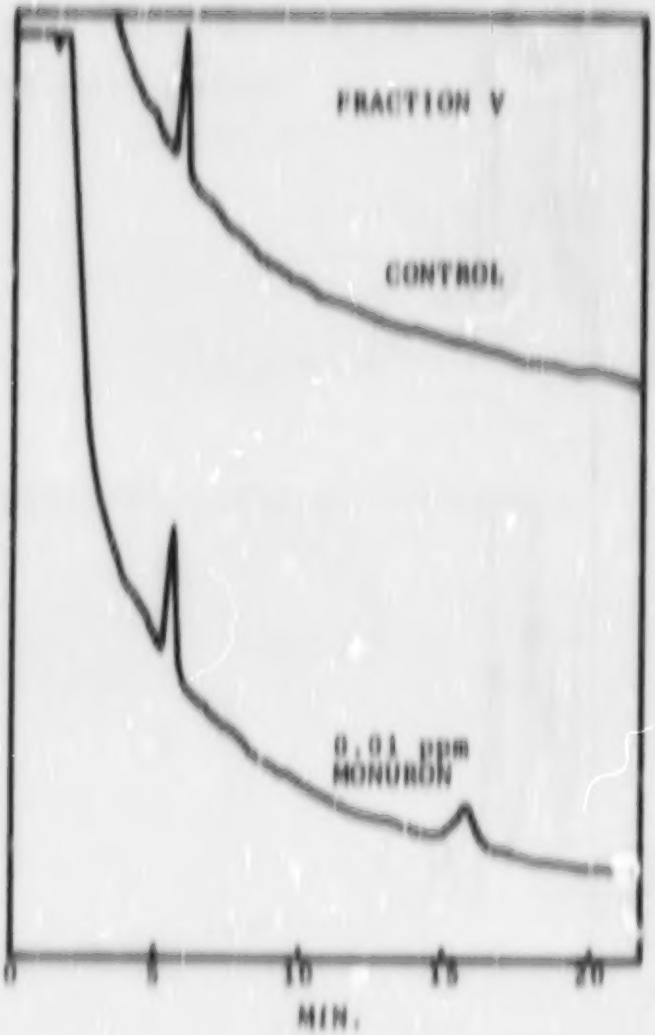


FIGURE 28 a

Chromatograms of PFBC-control (5 mg soil equivalent) and recovery (83%) of 0.01 ppm monuron from sandy soil. G.C. conditions as in Fig. 27 b.

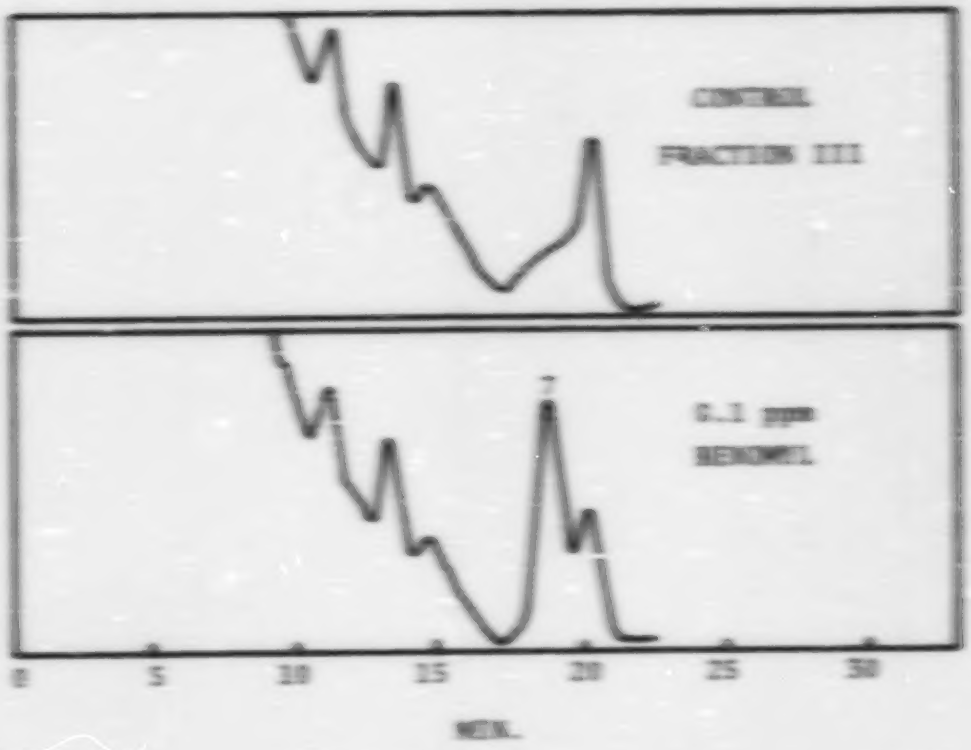


FIGURE 24 b

Chromatograms of PFBC-control (10 mg soil equivalent) and recovery (80%) of 0.1 ppm benomyl from sandy soil. (1.5% OV-17/1.95% OV-210, 220°C Att. 2 x 10[illegible].

Table 27. Average percent recoveries of phorate and parathion from sandy soil which were spiked at 0.01, 0.10, 1.00 and 10.00 ppm. Comparison of tumbling and soxhlet extraction methods (without cleanup, FPD).

	% recovery of pesticide from ppm spiked[1]			
	0.01 ppm	0.10 ppm	1.00 ppm	10 ppm
Tumbling				
Phorate	86.6	46.6	28.0	62.7
Parathion	81.0	71.5	37.6	67.3
Soxhlet				
Phorate	96.6	94.0	69.0	90.3
Paration	93.3	99.0	73.6	90.6

---

[1] Average percent recovery of triplicate samples.

particles were treated with trifluralin at 10 ppm, it was recovered from soil more readily (70.5-91.5% from all types of soils). The low recoveries of bromacil and endosulfan from sandy soil (Table 30) might be attributed to the higher percentage of organic carbon in the sandy soil.

2. Organophosphate pesticides. Average percent recoveries of seven organophosphate pesticides from crude sandy and sandy loam soil extracts are presented in Tables 32 and 33 respectively. Considerably lower recoveries were obtained for demeton treated sandy loam soil (68-82%) and sandy soil samples (60-73.3%). Average percent recoveries of other pesticides ranged from 73.3% (1 ppm malathion) to 103% (0.10 ppm azinphos ethyl) for sandy loam soil and 67% (0.01 ppm azinphos ethyl) to 102.6% (0.01 ppm methyl parathion) for sandy soil. When crude silty loam soil extracts of organophosphate pesticides were cleaned up by silica gel columns (Table 34), there were no significantly different recovery results than those non-cleaned up soil extracts (Tables 32 and 33). Therefore, it might be concluded that silica gel retained negligible amounts of the tested organophosphate pesticides. No major difference of percent recoveries from various soil types was observed (Tables 32-34).

Recoveries of some Organophosphate and Thermally Labile Nitrogen-Containing Pesticides from Soil by Polytron Extraction and Comparison of Results with Soxhlet Extraction.

Table 28. Soil Characterization[1]

Class and particle size distribution (%)	Soil Type[2] Sandy	 Sandy Loam	 Silt Loam
Sand (2-0.05 mm)	84.0	92.6	54.2
very coarse (2-1 mm)	0.1	3.1	0.0
coarse (1-0.5 mm)	3.7	4.8	0.1
medium (0.5-0.25 mm)	31.0	38.8	1.5
fine (0.25-0.10 mm)	42.5	41.0	33.0
very fine (0.10-0.05 mm)	6.7	7.9	13.6
Silt (0.05-0.002 mm)	12.0	1.5	24.3
Clay (0.002 mm)	4.0	5.9	19.9
Organic Carbon (percent)	2.64	0.23	0.33
pH			
H_2O (1:1)	4.8	5.2	4.7
$CaCl_2$ (0.01 M, 1:2)	3.7	4.4	4.3
KCl (1 N, 1:1)	3.5	4.2	3.8

[1] Soil samples were analyzed by Soil Characterization Laboratory, McCarty Hall, IFAS, University of Florida

[2] Sandy and sandy loam soil were collected from Gainesville, Florida, silt loam soil was provided by EPA.

Table 29. Average percent recoveries of halogenated pesticides from silty loam soil which were spiked at 0.01, 0.10, 1.00, and 10.00 ppm (Soxhlet extraction except where otherwise indicated, with silica gel column clean-up, EC detector).

Pesticide	% recovery of pesticide from ppm spiked[1]			
	0.01 ppm	0.10 ppm	1.00 ppm	10 ppm
Atrazine	87.3	79.0	90.0	89.3
Bromacil	79.7	85.8	63.0	76.3
Captan[3]	100.0	70.3	87.0	75.0
Chlorobenzilate	ND[2]	90.3	92.7	76.3
CIPC	84.0	70.3	77.0	92.0
Difolatan[3]	82.0	80.3	79.7	80.0
Endosulfan	94.7	68.0	95.0	104.0
Folpet[3]	100.0	92.7	96.0	85.0
Methoxychlor	108.0	86.3	96.2	95.2
PCNB	73.0	84.7	85.0	88.7
Perthane	85.0	67.0	101.7	85.3
Simazine	95.0	82.3	86.0	91.3
Trifluralin	80.0	67.0	83.7	71.3

[1] Average percent recovery of triplicate samples.

[2] ND = non-detectable.

[3] These pesticides reacted with methanol when 1:1 Benzene/Methanol was used for Soxhlet extraction; therefore, 200 ml of Benzene was used with tumbling extraction for the spiked soil samples for 1 hour.

Table 30. Average percent recoveries of halogenated pesticides from sandy soil which were spiked at 0.01, 0.10, 1.00 and 10.00 ppm (Soxhlet extraction with silica gel column cleanup, EC detector).

	% recovery of pesticide from ppm spiked[1]			
Pesticide	0.01 ppm	0.10 ppm	1.00 ppm	10 ppm
Atrazine	ND	60.0	84.7	92.7
Bromacil	95.7	61.3	56.0	74.5
Chlorobenzilate	100.0	85.7	91.0	97.2
CIPC	ND	92.7	82.3	99.7
Endosulfan	78.3	63.0	63.0	69.0
Methoxychlor	82.7	76.3	78.7	84.0
PCNB	69.0	73.0	88.0	95.9
Perthane	77.3	71.7	84.7	94.5
Simazine	91.0	77.5	110.0	92.3
Trifluralin	59.3	50.0	52.5	70.5

[1] Average percent recovery of triplicate samples.

Table 31. Average percent recoveries of halogenated pesticides from sandy loam soil which were spiked at 0.01, 0.10, 1.00, and 10.00 ppm (Soxhlet extraction with silica gel column cleanup, EC detector).

	% recovery of pesticide from ppm spiked[1]			
Pesticide	0.01 ppm	0.10 ppm	1.00 ppm	10 ppm
Atrazine	83.7	72.7	92.3	95.0
Bromacil	96.3	88.0	91.0	93.0
Chlorobenzilate	99.7	93.5	96.0	80.7
CIPC	106.0	88.0	61.0	85.0
Endosulfan	81.3	77.0	83.0	86.0
Methoxychlor	99.0	85.3	89.7	91.0
PCNB	70.0	76.0	77.0	92.5
Perthane	96.0	75.7	77.0	87.5
Simazine	97.0	79.0	87.2	97.0
Trifluralin	47.0	54.0	49.7	91.5

[1] Average percent recovery of triplicate samples.

Carbofuran was selected for use in evaluating a number of extraction parameters which included solvent type, ultrasonic power, and extraction time. Benzene-methanol (1:1) was found to be the best extracting solvent (Table 35). No significant difference was observed in the extraction of carbofuran from sandy soil by varying extraction time (30 to 90 sec. Table 36). However, the best recovery of carbofuran was obtained when a maximum motor speed of the Polytron was applied (Table 37). Later experience gained from this investigation suggested use of medium speed, because the Polytron generator head was being ground away excessively by soil particles.

Average percent recoveries of sixteen multi-class pesticides from sandy soil are presented in Table 38. Benzene-methanol (1:1) was selected as the solvent for general extraction, although methanol reacted with some pesticides during Soxhlet extraction (Table 39) and subsequently yielded poor or no recovery for carbamates, captan, folpet and Difolatan. Polytron extraction (Table 38) apparently overcame the problem and gave reasonably good recoveries for those pesticides at 0.01-0.1 ppm levels (captan, 64-76%; Difolatan, 89-100%; folpet, 88-121%). Other pesticides in Table 38 which could be detected at 0.01-0.1 ppm levels were: Avadex (70-75%), bux (89%), dicamba (77-66%), DNBP (74-75%), DNOC (60-67%), carbofuran (100%) and monuron (83-93%). Pesticides which could not be detected at 0.01-0.1 ppm levels but were detectable at 1 and 10 ppm levels were benomyl (61-69%), IPC (71-73%), Landrin -2,3,5 (80-81%), Landrin -3,4,5 (73-82%) and Zectran (76-85%). The only pesticide which

Table 32. Average percent recoveries of organophosphate pesticides from sandy soil which were spiked at 0.01, 0.10, 1.00, and 10.00 ppm (Soxhlet extraction without column cleanup, FPD detector).

Pesticide	% recovery of pesticide from ppm spiked[1]			
	0.01 ppm	0.10 ppm	1.00 ppm	10 ppm
Phorate	96.6	94.0	69.0	90.3
Parathion	93.3	99.0	73.6	90.6
Demeton	60.0	60.0	71.7	73.3
Malathion	70.0	81.0	90.3	93.3
Methyl parathion	102.6	93.3	82.0	88.3
Azinphos ethyl	67.0	71.7	93.3	99.7
Azinphos methyl[2]	86.0	101.7	100.7	92.3

[1] Average percent recovery of triplicate samples.

[2] With silica gel column cleanup.

Table 33. Average percent recoveries of organophosphate pesticides from sandy loam soil which were spiked at 0.01, 0.10, 1.00, and 10.00 ppm (Soxhlet extraction without column cleanup, FPD detector).

Pesticide	% recovery of pesticide from ppm spike[1]			
	0.01 ppm	0.10 ppm	1.00 ppm	10 ppm
Phorate	77.3	102.6	89.0	80.0
Parathion	99.0	84.6	75.3	81.0
Demeton	68.0	73.0	73.3	82.0
Malathion	92.6	84.0	73.3	80.6
Methyl parathion	102.6	83.0	84.0	72.6
Azinphos ethyl	83.3	103.0	102.0	92.0
Azinphos methyl	101.6	90.3	78.3	83.3

[1] Average percent recovery of triplicate samples.

Table 34. Average percent recoveries of organophosphate pesticides from silty loam soil which were spiked at 0.01, 0.10, 1.00, and 10.00 ppm (Soxhlet extraction, with silica gel column cleanup, FPD detector).

Pesticide	% recovery of pesticide from ppm spike[1]			
	0.01 ppm	0.10 ppm	1.00 ppm	10 ppm
Phorate	75.0	81.0	75.0	71.7
Parathion	93.0	101.0	85.7	86.0
Demeton	69.3	72.3	69.3	71.3
Malathion	95.7	96.7	96.7	101.0
Methyl Parathion	101.0	91.7	93.3	91.0
Azinphos ethyl	72.0	90.3	86.7	87.3
Azinphos methyl	77.7	91.0	89.3	78.3

[1] Average percent recovery of triplicate samples.

Table 35. Comparison of solvents for ultrasonic-polytron extraction of carbofuran (1 ppm) from sandy soil.

Solvent	mean % recovery of duplicate samples and deviation from mean
Acetone	71.5 ± 3.5
Acetonitrile	70.5 ± 0.5
Methanol	83.0 ± 2.0
Hexane	24.8 ± 5.5
Benzene	74.3 ± 3.8
Benzene-Methanol (1:1)	100 ± 0.0

GLC column and operating conditions: 4% SE-30/6% OV-210, 200°C, Att. = 4 x 10^2 (PFPA derivatisation, EC).

Soil and solvent: 50 g air-dried sandy soil extracted by 100 ml x 2 of solvent and washed with 50 ml x 2.

Polytron: 1 min x 2, maximum speed.

could not be detected even at the 10 ppm level, was amitrole.

Due to high water solubility of amitrole, most conventional solvents used for extraction were water or an ammonium hydroxide-glycol (5+20) mixture (26). Groves and Chough (26) suggested that polar compounds like amitrole may be bound in the clay-organic matter ion exchange systems similar to the binding of nutrient ions; that entrapment between atom-layers of expandable lattice clays may occur; that insoluble compounds or complexes may be formed; and that adsorption may occur due to electrostatic or residual valence forces. The undetectability of benomyl at low residue levels (0.01-0.1 ppm) was attributed to the low sensitivity of the PFBC derivative and the high background interference of the PFBC reagent as mentioned before. IPC, Landrins, and Zectran could not be detected at low levels (0.01-0.1 ppm) (Table 38) mainly due to the interference peaks of the PFPA derivatives and their short retention times which appeared in the same regions as most underivatized soil coextractives.

The Soxhlet extraction (Table 39) procedure prevented the complete recoveries of captan, folpet, Difolatan, carbamate and substituted urea compounds due to pesticides-methanol interaction or thermal degradation under prolonged (12 hr) elevated temperature. However, better recoveries were obtained with Soxhlet extraction than with the Polytron for the following heat stable pesticides: Avadex (75-100 vs. 70-75), dicamba (86-97 vs. 77-86), DNBP (91-100 vs. 74-75), DNOC (99-104 vs. 60-67).

Table 36. Effect of Polytron extraction time on recovery of carbofuran from sandy soil.*

Extraction time, sec	Mean % recovery of duplicate samples and deviation from mean
30 x 2	71.3 ± 0.5
60 x 2	77.0 ± 1.0
90 x 2	72.0 ± 2.0

*GLC, column and conditions: same as Table 34.

Soil and extraction solvent: 50 g air-dried soil extracted by 100 x 2 ml Benzene-MeOH (1:1) and washed with 50 x 2 ml.

Polytron speed: 50% of maximum.

Table 37. Effect of Polytron motor speed on recovery of carbofuran from sandy soil.

Speed, % of maximum	mean % recovery of duplicate samples and deviation from mean
30	71.5 ± 6.5
50	71.5 ± 6.5
70	75.0 ± 1.0
100	100 ± 0.0

GLC, column and conditions: same as Table 34.

Soil and extraction solvent: same as Table 35.

Polytron extraction time: 1 min x 2.

Table 58. Average percent recoveries of pesticides from sandy soil samples (triplicate) which were spiked at 0.01 and 0.10 ppm (Polytron extraction with silica gel column cleanup, EC detector).[1]

Pesticide	% recovery of pesticide from ppm spiked[2]	
	0.01 ppm	0.10 ppm
Amitrole[3]	(undetectable)	(undetectable)
Avadex	70.0	75.0
Benomyl[3]	(69.3)	(61.3)
Bux	undetectable	89.5
Captan[4]	76.0	64.3
Dicamba[5]	86.0	77.0
Difolatan[4]	89.3	100.0
DNBP[5]	75.3	74.0
DNOC[5]	67.3	60.0
Carbofuran	undetectable	100.0
Folpet[4]	88.3	101.0
IPC	(72.7)	(70.7)
Landrin-2,3,5	(79.7)	(81.0)
Landrin-3,4,5	(82.0)	(73.3)
Monuron	83.0	93.0
Zectran	(85.0)	(76.0)

[1] See Tables 21 and 22 for RRT, RPA and GLC operating conditions

[2] Parentheses imply undetectable at 0.01 and 0.10 ppm levels, subsequent attempts for 1 and 10 ppm were conducted and recorded in parentheses respectively.

[3] Amitrole and benomyl were derivatized before silica gel column cleanup.

[4] Solvents were evaporated by standing in a hood at room temperature (other pesticides by Kuderna-Danish concentrator).

[5] Analyzed in crude extract.

Table 59. Average percent recoveries of pesticides from sandy soil samples (triplicate) which were spiked at 0.01, and 0.10 ppm (Soxhlet extraction, with silica gel column cleanup, EC detector).

Pesticide	% recovery of pesticide from ppm spiked	
	0.01 ppm	0.10 ppm
Amitrole	(undetectable)	(undetectable)
Avadex	100.0	73.3
Benomyl	undetectable	85.0
Bux	64.3	39.3
Captan	0.0	0.0
Dicamba	85.5	97.0
Difolatan	0.0	0.0
DNBP	90.7	100.0
DNOC	104.3	99.2
Carbofuran	undetectable	48.3
Folpet	0.0	0.0
IPC	(60.1)	(65.0)
Landrin-2,3,5	(57.0)	(63.3)
Landrin-3,4,5	(50.3)	(64.3)
Monuron	50.3	37.0
Zectran	30.0	33.3

In the Soxhlet extraction, benomyl was converted to Methyl N-(2-benzimidazolyl) carbamate (MBC) and 2-aminobenzimidazole (2-AB) more readily than in the Polytron procedure. The better recoveries with the Soxhlet procedure (85% at 0.1 ppm vs. 61-69% at 1-10 ppm) reflected the detection of the degradation products of benomyl, i.e., PFBC-MBC and PFBC-2AB.

Average percent recoveries of five organophosphate pesticides by Polytron and Soxhlet extraction from sandy soil are presented in Tables 40 and 41 respectively. High percent recoveries were obtained for most pesticides (82-103%) by the Polytron extraction except for methomyl and Temik (Table 40). The inability to recover methomyl was partly due to loss during Kuderna-Danish evaporation, whereas the disappearance of Temik might be due to its strong binding with soil particles. There was no evidence of any breakdown or alteration of Temik or other pesticides by the Polytron extraction which agrees with the findings of Johnsen and Starr (28).

The above indefinite conclusion led us to further investigate the disappearance of these two compounds. No acceptable recovery results could be obtained for these compounds by room temperature solvent evaporation or by mixing the concentrated control sandy soil sample with Temik and methomyl standards. Pesticide standard peaks also disappeared when they were injected simultaneously from the same syringe together with the same amount of control soil extract. All the above evidence led us to believe that carbamoyl pesticides reacted with soil coextractives to produce other compounds under elevated temperature in the GLC system.

Simultaneous analysis of forty multi-class pesticides in sandy soil

Duplicate sandy soil samples were spiked with 0, 0.01 and 0.1 ppm of the forty multi-class pesticides listed in Table 1 and analyzed for their recoveries simultaneously. Table 42 presents the results of fourteen EC-sensitive pesticides. Excellent recoveries were obtained for most pesticides at 0.01 ppm level (90-116%) except for Perthane, chlorobenzilate, captan, folpet and Difolatan. Perthane was masked by the more responsive endosulfan while chlorobenzilate suffered from interference by coextractives from sandy soil. Captan, folpet and Difolatan reacted with methanol during the Soxhlet extraction procedure. At 0.1 ppm, all the halogenated pesticides gave excellent recoveries (86-115%) except for atrazine and simazine (72%).

Data in the last column of Tables 42, 43, and 44 are derived from 0.1 ppm equivalent of pesticides (without soil) and underwent the entire procedure used in the extraction of pesticides from soil samples. The original purpose was to determine whether low recovery results were caused by the analytical procedure itself or because of the soil binding. No endosulfan could be recovered when it was heated in the Soxhlet extractor without soil for 12 hours in contrast to 95% recovery of the same amount of pesticide spiked in soil (0.1 ppm). Considerably lower recoveries were obtained from non-soil samples as compared to their corresponding soil samples, i.e., PCNB (87 vs. 93%), trifluralin (73 vs. 86%), chlorobenzilate (80 vs.

Table 40. Average percent recoveries of pesticides from sandy soil samples (triplicate) which were spiked at 0.01 and 0.10 ppm (Polytron extraction with silica gel column cleanup, FPD; P-mode).[1]

Pesticide[4]	% recovery of pesticide from ppm spiked[2]	
	0.01 ppm	0.10 ppm
Azodrin	83.0	81.7
Diazinon	93.3	87.9
Dimethoate	102.7	98.3
Dursban	94.0	96.7
Methomyl[3]	(undetectable)	(0.0)
Phosphamidon	88.7	90.3
Temik[3]	(undetectable)	(13.0)

[1] See Table 9 for RRT, RPA and GLC operating conditions.

[2] See footnote of Table 18 for explanation.

[3] FPD operated on S-mode, see Figure 21 for analytical curves.

[4] Solvents were evaporated by Kuderna-Danish concentrator.

Table 41. Average percent recoveries of pesticides from sandy soil samples (triplicate) which were spiked at 0.01 and 0.10 ppm (Soxhlet extraction with silica gel column cleanup, FPD; P-mode).

Pesticide	% recovery of pesticide from ppm spiked	
	0.01 ppm	0.10 ppm
Azodrin	91.0	98.0
Diazinon	61.0	99.5
Dimethoate	91.0	102.2
Dursban	66.0	104.0
Methomyl	0.0	0.0
Phosphamidon	76.0	100.0
Temik	0.0	0.0

Table 42. Multi-residue analysis of fourteen EC-sensitive pesticides in duplicate sandy soil samples which were spiked at 0.01, and 0.1 ppm of forty multi-class pesticides in Table 1 (Soxhlet extraction, 4% SE-30/6% OV-210, 205°C, Att. : 2 x 10^2, N_2 flow = 67 ml/min, Tracor 222, EC).

Pesticides eluted in silica gel fractions	% recoveries from ppm spiked		
	0.01 ppm	0.10 ppm	(0.10 ppm)[1]
Hexane			
PCNB	116	93	87
60% Benzene/Hexane			
Avadex	110	115	107
Perthane	undetectable	93	80
Trifluralin	91	86	73
Methoxychlor	107	88	88
Endosulfan	94	95	0[2]
5% Acetonitrile/benzene			
CIPC	"———— undetectable	————"	
Captan	underwent structural transformation in the Soxhlet extracting solvents (benzene/methanol, 1:1)		
Folpet			
Difolatan			
Chlorobenzilate	undetectable	96	80
10% Acetonitrile/benzene			
Atrazine	90	72	64
Simazine			

Table 42 continued.

Pesticides eluted in silica gel fractions	% recoveries from ppm spiked		
	0.01 ppm	0.10 ppm	(0.10 ppm)[1]
20% Acetonitrile/benzene			
Bromacil	95	93	95

---

[1] 0.1 ppm equivalent of pesticides without soil underwent the same extracting procedure (Soxhlet), evaporation (Kuderna-Danish) and cleanup (silica gel).

[2] Loss due to absence of Keeper.

96%). Identical results were obtained for methoxychlor (88%) and bromacil (95%).

Table 43 presents the results of multi-residue analysis of twelve FPD-P sensitive pesticides in duplicate sandy soil samples which were spiked at 0, 0.01, and 0.10 ppm of forty multi-class pesticides. At 0.01 ppm, pesticides which were recovered above 70% were: methyl parathion (77%), parathion (76%), malathion (100%), dimethoate (91%), phosphamidon (76%) and Asodrin (91%). Those recoveries below 70% were: phorate (37%), Dursban (56%), diazinon (41%), and demeton (40%). At 0.1 ppm, all twelve OP pesticides were recovered at more than 78% (79-100%). The 0.1 ppm of the non-soil samples (last column of Table 43) gave very poor recoveries except for parathion (71%) and Asodrin (76%). The low recoveries (5-53%) of other OP pesticides, i.e., phorate (48%), methyl parathion (37%), diazinon (45%), malathion (32%) demeton (5%) and dimethoate (53%), might be attributed to their thermal degradation on the glass surfaces of the Soxhlet extractor in the absence of soil coextractives. Soil coextractives might act as protective agents in preventing pesticide breakdown on the glass surface. It is already known in GLC that regular injection of sample extracts or silanizing reagents will help prevent the degradation of some relatively heat-labile compounds.

Table 44 presents the results of multi-residue analysis of twelve nitrogen containing pesticides in duplicate sandy soil samples which were spiked at 0, 0.01 and 0.10 ppm. At 0.01 ppm, good recoveries were obtained from DNOC (73%) and DNBP (103%).

Table 43. Multi-residue analysis of twelve FPD-P sensitive pesticides in duplicate sandy soil samples which were spiked at 0.01 and 0.10 ppm of forty multi-class pesticides in Table 1 (Soxhlet extraction, 4% SE-30/6% OV-210, 200° C, Att. = 16 x 10^{-6}, N_2 flow = 50 ml/min, Varian 1200, FPD-P).

Pesticides eluted in silica gel fractions	% recoveries from ppm spiked		
	0.01 ppm	0.10 ppm	(0.10 ppm)[1]
60% benzene/hexane			
Phorate	37	94	48
Dursban	66	79	72
Methyl parathion	77	100	37
Parathion	76	88	71
5% acetonitrile/benzene			
Diazinon	41	90	45
Malathion	100	106	52
10% acetonitrile/benzene			
Demeton	40	86	2
20% acetonitrile/benzene			
Dimethoate	91	98	53
100% acetonitrile/benzene			
Phosphamidon	76	100	2
Azodrin	91	100	76

[1,2] See footnotes of Table 42.

Note: Temik and methomyl were unrecoverable (detected by FPD-S).

Table 44. Multi residue analysis of twelve nitrogen containing pesticides in duplicate sandy soil samples which were spiked at 0.01, and 0.10 ppm of forty multiclass pesticides in Table 1 (Soxhlet extraction, 4% SE-30/6% OV-210, 205° C, Att. = 2 x 10^2, N_2 flow = 67 ml/min; Tracor 222, EC).

Pesticides eluted in silica gel fractions	% recoveries from ppm spiked		
	0.01 ppm	0.10 ppm	(0.10 ppm)[1]
Without column cleanup			
Dicamba (DAM)	59	103	18
DNOC (DAM)	73	112	35
DNBP (DAM)	103	117	100
Benomyl (PFBC)	undetectable	90	80
5% Acetonitrile/benzene			
2,3,5-Landrin (PFPA)		undetectable	
3,4,5-Landrin (PFPA)			
Zectran (PFPA)			
IPC (PFPA)			
Bux (PFPA)			
20% Acetonitrile/benzene			
Carbofuran (PFPA	"——	undetectable	——"
Monuron (PFBC)	50	52	38
100% Acetonitrile/benzene			
Amitrole (PFBC)	"——	undetectable	——"

---

[1] See footnotes of Table 41.

Poor recoveries were obtained for dicamba (59%) and monuron (50%). Pesticides which were undetectable at all concentrations were: Landrins, Zectran, IPC, bux, carbofuran, and amitrole. Benomyl was recoverable at 0.1 ppm (90%) while monuron gave poor results (52%) at this level. Excellent results were obtained for dicamba (103%), DNOC (112%), and DNBP (117%). Poor or no recoveries (below 38%) were observed for most of these pesticides in the non-soil samples (last column of Table 43) except for DNBP and benomyl.

Conclusion

Aside from a rapid deterioration of the Polytron generator rotor and bearings, it offers a real advantage in extracting multiclass pesticides from soil compared to either Soxhlet extraction or tumbling. More specifically, of the forty compounds studied, the following, which could be successfully extracted from soil with the Polytron, could not be extracted with the Soxhlet due to degradation: CIPC, captan, folpet, Difolatan, carbofuran, Zectran, bux and benomyl. Amitrole could neither be extracted by Soxhlet nor Polytron. It was not determined whether methomyl or Temik could be extracted by the Polytron (see part II for confirmation of this) since they could not be gas chromatographed in the presence of soil coextractives. Dicamba could not be eluted from the silica gel intact, however, it could be if it were methylated prior to chromatography. DNOC also could not be eluted at residue levels, however, it could be determined in the crude extract after methylation. See Fig. 29 for a summary of the procedure utilizing the Polytron.

Forty Multiclass Pesticides in Soil (50 g)[5]

Polytron
100 ml benzene/methanol (1:1)(x2)
wash with 50 ml (x2)

benzene/methanol soil extract
evaporate to dryness

Silica gel chromatography: 16 g (14 g Na_2SO_4)

Fraction I
50 ml hexane

EC PCNB

Fraction II
50 ml 40% B/H

EC trifluralin
Perthane
methoxychlor
endosulfan
Avadex
DNBP (DAM)[3]
dicamba (DAM)[1]

FPD
Dursban
phorate
methyl parathion
parathion

Fraction III
50 ml 5% A/B

EC
CIPC
captan
folpet
Difolatan
chlorobenzilate
benomyl (PFBC)[2]
DNOC (DAM)[1]
bux (PFPA)
IPC (PFPA)[4]
Zectran (PFBC)[4]
2,3,5-Landrin (PFPA)[4]
3,4,5-Landrin (PFPA)[4]

FPD
diazinon
malathion
azinphos methyl
azinphos ethyl

Fraction IV
50 ml 10% A/B

EC
atrazine
simazine
carbofuran (PFPA)

FPD
demeton

Fraction V
50 ml 20% A/B

EC
bromacil
monuron (PFBC)

FPD
phosphamidon
dimethoate

Fraction VI
50 ml Acetonitrile

FPD
Azodrin

Figure 29. Procedure for detection of forty pesticides in soils (A/B = Acetonitrile/benzene).

---

[1] Derivative made prior to silica-gel cleanup.
[2] Detectable only at 1 ppm level.
[3] Analyzed in crude extract.
[4] Can only be analyzed at 1.0 ppm and higher.
[5] Detectable at 0.01 or 0.1 ppm unless otherwise specified.

References (Part I)

1. Argauer, R. J., J. Agr. Food Chem. 17, 888 (1969).
2. Bowman, M. C., Beroza, M. J., J.A.O.A.C. 50, 926 (1967).
3. Crosby, D. G., Bowers, J. B., J. Agr. Food Chem. 16, 839, (1968).
4. Shafik, M. T., Bradway, D., Biros, F. J., Enos, H. F., J. Agr. Food Chem. 18, 1174 (1970).
5. Khalifa, S., Mumma, R. O., J. Agr. Food Chem. 20, 632, (1972).
6. Mills, P. A., Onley, J. H., Gaither, R. A., J.A.O.A.C. 46, 186 (1963).
7. Johnson, L., J.A.O.A.C. 48, 668 (1965).
8. Porter, M. L., Young, S. J., Burke, J. A., J.A.O.A.C. 53, 1300 (1970).
9. Wessel, J., J.A.O.A.C. 52, 172 (1969).
10. Carr, R. L., J.A.O.A.C. 54, 525 (1971).
11. Chiba, M., Residue Reviews 30, 63 (1969).
12. Sherma, J., Shafik, T. M., Arch. Environ. Contam. Toxicol. 3, 55 (1975).
13. Moye, H. A., J. Agr. Food Chem. 23, 415 (1975).
14. Damico, J. N., Benson, W. R., J.A.O.A.C. 48, 344 (1965).
15. Fishbein, L. Zielinski, W. L., J. Chromatog. 20, 9 (1965).
16. Moye, H. A., J. Agr. Food Chem. 19, 452 (1971).
17. Kawahara, F. K., Anal. Chem. 40, 1009 (1968).
18. Yip, G., Howard, S. F., J.A.O.A.C. 51, 24 (1968).

19. Kushy, K. T., Kaiser, D. G., Van Der Slik, A. L., J. Chrom. Sci. 13, 97 (1975).

20. Nash, R. G., J.A.O.A.C. 57, 1015 (1974).

21. Stewart, F. H. C., Aust. J. Chem. 13, 478 (1960).

22. Roth, M., Analy. Chem. 43, 880 (1971).

23. Carey, W. F., Kenneth, H., J.A.O.A.C. 53, 1296 (1970).

24. Zehner, J. M., J. Chrom. Sci. 14, 348 (1976).

25. Knaak, J. B., Tallant, M. J., Sullivan, L. J., J. Agr. Food Chem. 14, 573 (1966).

26. Groves, K., Chough, K. S., J. Agr. Food Chem. 19, 840 (1971).

27. Fung, K. K. H., J. Agr. Food Chem. 23, 695 (1975).

28. Johnsen, R. E., Starr, R. I., J. Agr. Food Chem. 20, 48 (1972).

29. Brown, B., Goodman, J. E., "High Intensity Ultrasonic" Van Nostrand, New York, N.Y., (1965) pp. 196-220.

Extension of Multi-residue Methodology. II. Dynamic Fluorogenic Labelling Detector for Carbamates.

H. Anson Moye and Gordon Cash

Introduction

Many pesticides now in common use are formally derivatives of methylcarbamic acid, i.e. carbamate esters or carbamoyl oximes. These pesticides are somewhat polar and extremely heat labile, making them poorly suited for conventional column cleanup techniques and separation by gas-liquid chromatography. Several approaches have been used to attempt to overcome these difficulties. One method is to form chemical derivatives which improve volatility and thermal stability (1,2). This method is often satisfactory if the analysis is directed toward only one compound at a time. Colorimetric procedures involving coupling with diazonium salts also have been utilized in similar situations. Many reports of multi-residue methods for carbamates have described separation by thin-layer chromatography and quantitation by various methods including chromogenisis, enzyme inhibition and, in situ fluorometry.

It would appear that high pressure liquid chromatography (HPLC), since it can achieve high efficiency separations at ambient temperatures could theoretically obviate the themal instability problem associated with the carbamates. However, detection at the nanogram level normally required for sub-part-per-million analysis in foods and environmental samples, would seem to be unlikely with commercially available detectors. Preliminary experiments in this laboratory have demonstrated this to be the case that the carbamates as a class neither absorb U.V.-visible radiation nor fluoresce sufficiently (with the possible exceptions of carbaryl and benomyl) to allow for simple and straight forward extraction, cleanup and detection schemes.

Recent success with post-column fluorogenic labelling of amino acids following a HPLC separation has offered encouragement that this approach could be used for the analysis of the carbamates in soil (3). The fluorogen used in that study was Fluram^{R}, (4-phenylspiro [furan-2(3H), 1-phthalan]-33-dione) (4). This reagent is non fluorescent, nor is its hydrolysis product.

Another promising fluorogenic reagent seemed to be o-phthalaldehyde (o-phthalic-dicarboxaldehyde) reported by Roth (5) to be extremely sensitive for primary amino acids. Like Fluram it reacts almost instantaneously, is itself non-fluroescent, nor are its hydrolytic products. Unlike Fluram, however, it is stable in protic solvents. Fluram is required to be handled in aprotic solvents, such as acetone.

Additionally, two other fluorogens specific for primary amines were investigated during the contract period, NBD-Cl

(4-chloro-7-nitrobenzo-2,1,3-oxadiazole) and dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride).

Eight commercially available, and widely used, carbamates were chosen for this study: Lannate^{R} (methomyl), Matacil^{R} (aminocarb), Temik^{R} (aldicarb), Baygon^{R}, Furadan^{R} (carbofuran), Sevin^{R} (carbaryl), Mesurol^{R} and Zectran^{R}. Lannate and Temik are actually carbamoyl oximes but can be effictively hydrolyzed to methylamine and hence should be detectable under the proposed system.

The three objectives for this HPLC portion of contract work, as specified in the contract were: (1) determine the optimum HPLC separation parameters for the above listed pesticides that would be compatible with the dynamic fluorogenic labelling detector, (2) determine the optimum parameters for the dynamic fluorogenic labelling detector and (3) demonstrate the sensitivity and selectivity of the combined column-detector system for the analysis of the listed carbamates in soils.

Additionally, a brief comparison was made between the assembled high pressure liquid chromatograph and a commercially available liquid chromatograph, the American Instrument Company Aminalyzer^{R} (Silver Springs, MD).

Experimental

Apparatus and Reagents

1. Two Waters model 6000 solvent delivery systems (pumps) were programmed with a Waters model 660 solvent

programmer (Waters Associates, Inc., Milford, Mass).

2. Sample injection valve Chromatronix HPSV with 25 ul sample loop (Spectra Physics, Berkeley, CA).

3. Columns, analytical HPLC, 30 cm x 4 mm I.D.; Waters: μ Carbohydrate. 25 cm x 4 mm I.D.: Dupont; Zorbax-ODS. 1 m x 4 mm I.D.: Dupont; ODS, ETH, SAX. Reeve Angel: Pellidon. Applied Sciences: Vydac Reverse Phase.

4. Kel-F "T"s (2), Laboratory Data Control (Riviera Beach, FL), model CJ3031.

5. Tubing, Teflon^{R}, 0.062 in O.D. x 0.02 in I.D., Dixon Medical Products (Thornton, PA). Cat. No. 6-8-112.

6. Detector, U.V. absorption, 254 nm, Waters.

7. Water bath, Haake, model FK.

8. Reagent pumps (2), Milton Roy model 196.

9. Fluorometer American Instrument Co., model 1258, with model B16-63019 flow through cell and model 416-993 mercury-xenon lamp. Excitation, 315 nm; emission, 435 nm (for o-phthalaldehyde). Slits, 4 mm. Scale 1.

10. Recorder, Soltec model 211, 50 mv.

11. Solvents: scintillation grade dioxane, deionized water, pH 10 0.05 M borate buffer, 0.02 M NaOH.

12. o-phthalaldehyde (OPA o-phthalicdicarboxaldehyde), Aldrich. Stock solutions were made by dissolving 1 g. in 10 ml of ethanol adding 1 ml of mercaptoethanol and diluting with buffer to volume.

13. Aminalyzer, American Instrument Co., equipped with

a Waters μC_{18} column.

14. Carbamate pesticides obtained from E.P.A., Research Triangle Park, NC.

HPLC Column Study

Two types of columns were evaluated, reverse phase and adsorption, at either of two particle sizes, 10 μ or 37 μ diameter. Those 37 μ reverse phase columns evaluated were: ODS, ETH, SAX, Vydac and Pellidon. The 10 μ reverse phase columns were: μC_{18}, μCN, μNH_2, μCarbohydrate and Zorbax-ODS. Only one adsorption column was evaluated, the μPorasil. Notice that the SAX column is normally a strong anion exchange column, but in this case was evaluated in the reverse phase mode.

The adsorption mode for the separation of the carbamates was immediately discarded after severe tailing was noted for isooctane-methanol or isooctane-chloroform mobile phases. This could not be overcome by water saturation of the mobile phases or by deactivation of the silica by water impregnation.

Much effort was expended in attempting an isocratic separation in the reverse phase mode using the 254 nm absorption detector and ug amounts of carbamates. Several columns were immediately discarded as being unsatisfactory, either because no reasonable separations were achieved or because too little retention was observed: ETH, SAX, Vydac Reverse Phase and Pellidon. The Zorbax-ODS column exhibited over 5000 psig back pressure, causing the sample injection valve to leak and therefore was discarded as a candidate.

Exhibiting about 5000 theoretical plates, the μC_{18} column appeared to be the most satisfactory giving adequate separation of the carbamate mix (Maticil was not included in this early work, Fig. 1), with 40% acetonitrile--water. At this percentage acetonitrile Baygon and carbofuran are not fully separated, although they can be easily quantitated; decreasing the acetonitrile percentage to 25% gave a complete separation at the baseline, their retentions being 29 and 35 minutes respectively. A flow of one ml/min was used throughout this work and was found to be nearly optimum while providing for rapid analyses.

The μCN column also performed well, giving about 2000 theoretical plates for a 1 foot x 4 mm I.D. column. However, at high percentages of acetonitrile (>20%) it could not satisfactorily separate Baygon and carbofuran. By operating this column at 12½% acetonitrile - 87½% water Lannate, Temik, Baygon and carbofuran could be separated quite well (Fig. 2).

By using the 12½% acetonitrile mobile phase the ODS column separated Sevin, Zectran and Mesurol quite well. The early eluters, while not shown (Fig. 3) elute immediately after the injection solvent. The Mesurol response shown is actually that of a conversion product produced by heating at 80°C for 15 min in ethanol; unchanged, it elutes too close to Zectran for adequate separation.

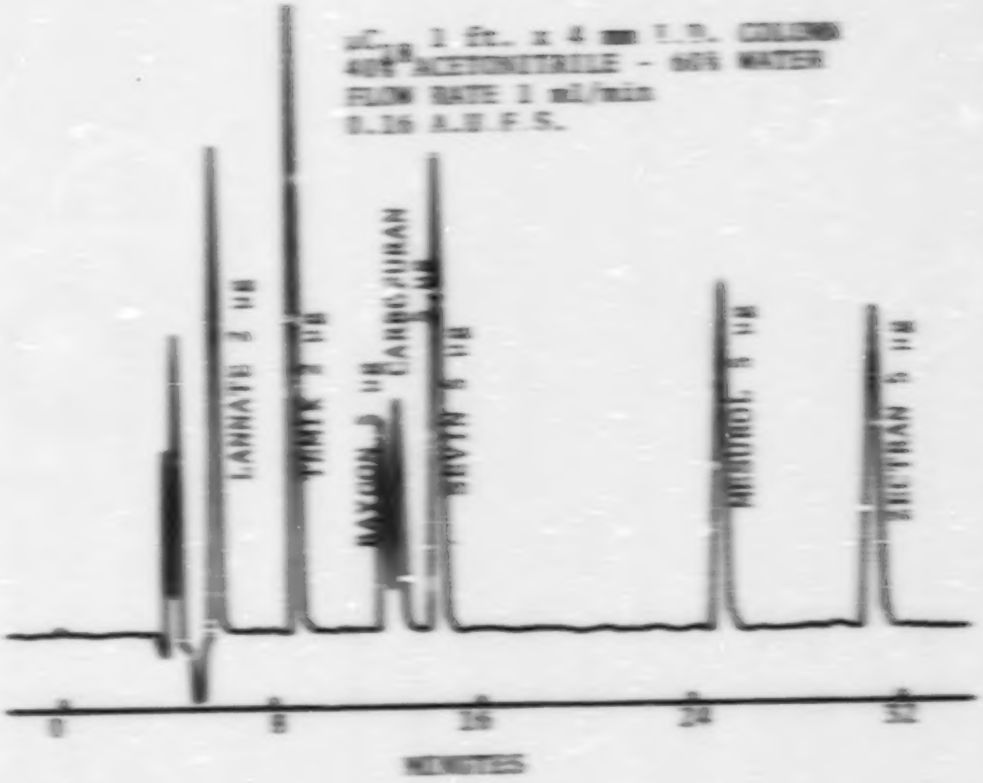


FIG. 1

Isocratic separation of six carbamate mix on μC_{18}, 40% acetonitrile - 60% H_2O.

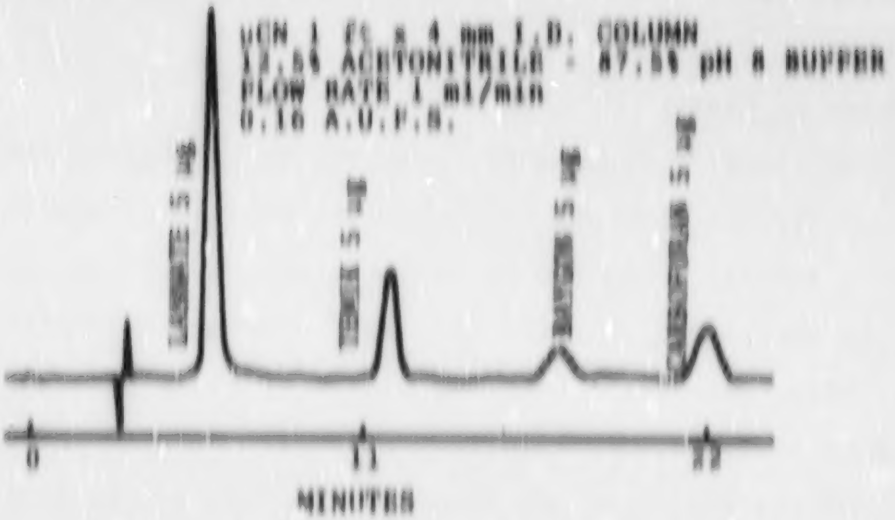


FIG. 2

Isocratic separation of early eluting carbamates on µCN, 12.5% acetonitrile - 87.5% H_2O.

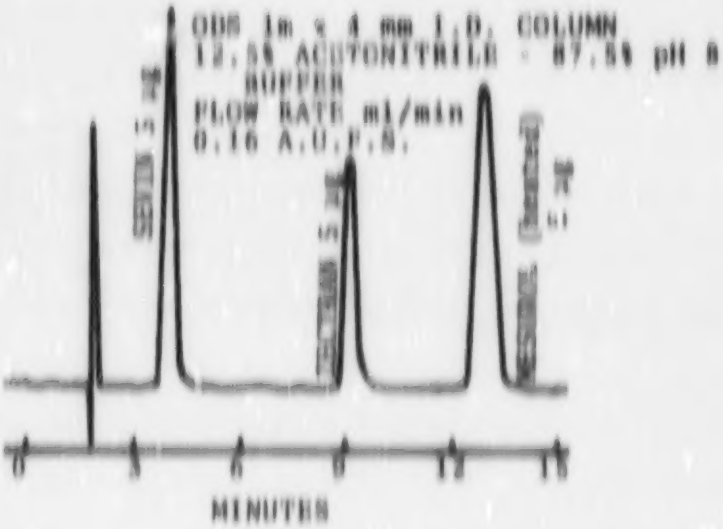


FIG. 3

Isocratic separation of late eluting carbamates on ODS, 12.5% acetonitrile - 87.5% H_2O.

The μ NH_2 and μ Carbohydrate columns provided poor separations for all of the carbamates and were considered unusable.

Studies on Fluram

Of the four fluorogenic reagents to be examined it became obvious that NBD-Cl, which requires a non-aqueous reaction medium, would not be satisfactory, since separations on the adsorption column (μ Porasil) were unachievable.

Also, dansyl chloride was given low priority, since the reagent itself fluorcesces considerably.

Of the remaining two reagents, Fluram was chosen for examination first, unfortunately so.

A. Static Measurements. Since Fluram is unreactive toward secondary amines in general, it was expected that it would not react with the intact esters of methylcarbamic acid. This was verified experimentally by tests with Sevin and Lannate. Weigele *et al* (3), however, reported that the secondary amino acids, proline and sarcosine, were rendered reactive toward Fluram by preliminary treatment with aqueous bromine or N-chlorosuccinimide under strongly acidic conditions (pH 1).

By treating Sevin with N-chlorosuccinimide (or N-bromosuccinimide, 10^{-3}M at 25°C for 2 hours) we were unable to observe fluorescence by adding Fluram (1.5×10^{-4}M) after buffering to pH9 with borate buffer. However, both Br_2 and I_2 at 10^{-3}M could produce maximum fluorescence by incubating for 100 minutes before buffering and reacting with Fluram. Linear analytical curves for all the carbamates were obtined from 1 to 10 nanomoles/ml of final solution. Table 1 gives the lower limit of detection (LLD) in nanomoles per ml of each carbamate, assuming a signal to noise ratio of 3:1.

Table 1

Carbamate	LLD (nanomoles/ml)
Carbofuran	0.95
Sevin	0.40
Zectran	0.50
Lannate	2.7
Temik	0.75
Baygon	1.5
Mesurol	0.71

B. Dynamic Measurements. A dynamic system was assembled separately from the column to simplify optimization of parameters unrelated to carbamate separation. A Harvard pulseless peristaltic pump was chosen for this early work in an effort to minimize noise from the fluorometric cell (flow through) as reagents were metered. Column effluent was simulated by passing an additional channel through the peristaltic pump. Zectran was used to optimize this system because it gave the greatest response in the static system.

The dynamic system was assembled with a view toward simulating the manual operations performed with the static system. Unfortunately, the sensitivity of the dynamic system never approached that of the static system and was a result of a combination of three factors: (1) since Fluram must be pumped in the aprotic solvent, acetone, no fully elastic tubing could be found which did not rapidly deteriorate, (2) since rapid mixing is essential for the Fluram to provide maximum response when metered (in acetone) into the buffered solution of primary amine, no fully satisfactory mixing device could be designed which did not add considerable band spreading and, (3) since complete mixing immediately upstream of the fluorometric cell could not be accomplished, considerable noise, due to a Schlieren effect, could not be eliminated.

Changing the solvent to dioxane did not improve the situation, nor did a series of elaborate in-line, mixing devices, the description of which is irrelevent here. Consequently after nine months effort with Fluram it was abandoned and the remaining three months given to studying o-phthalaldehyde.

Studies on o-Phthalaldehyde

A. Static Measurements. With only three months remaining in the contract period allotted to the HPLC work (no HPLC was to be done the second year of the contract period) it was decided to go immediately to a dynamic system and perform a rough optimization, based on the data of Roth (5).

It was found (see next section, Dynamic Studies on o-phthalaldehyde) that flows for the liquid chromatograph with the dynamic fluorogenic labelling detector, as well as solvent and reagent compositions, were optimum under the following conditions: (1) column flow 25 to 40% dioxane-H_2O, 1 ml/min (2) hydrolysis reagent, 0.02N NaOH, 0.4 to 0.5 ml/min., (3) OPA reagent, 1 g./l., 1.0 to 1.3 ml/min. By simulating these conditions in a Teflon-capped culture tube a study was made to determine the extent of hydrolysis occurring in the 35 foot hydrolysis coil.

To 2 ml of 25% dioxane - H_2O in the culture tube the appropriate carbamate was added to 10 µl of dioxane to give 10^{-6}M final concentration; then 1 ml of 0.02N NaOH was added. The tube was capped and incubated at 73°C in a water bath, after which it was removed, cooled rapidly to room temperature and reacted with 1 ml of the OPA reagent (1 g/l + 1 ml mercaptoethanol). The tube was swirled and read immediately at 336 and 454 nm in the fluorometer. Figures 4 through 7 show the results of the various hydrolysis reactions. Note that even after 30 minutes the fluorescent intensity is still climbing for all the carbamates, denoting incomplete hydrolysis at the intermediate times. When the fluorescence, for any of them, at 30 min is compared with that at 2.2 min (the residence time within the

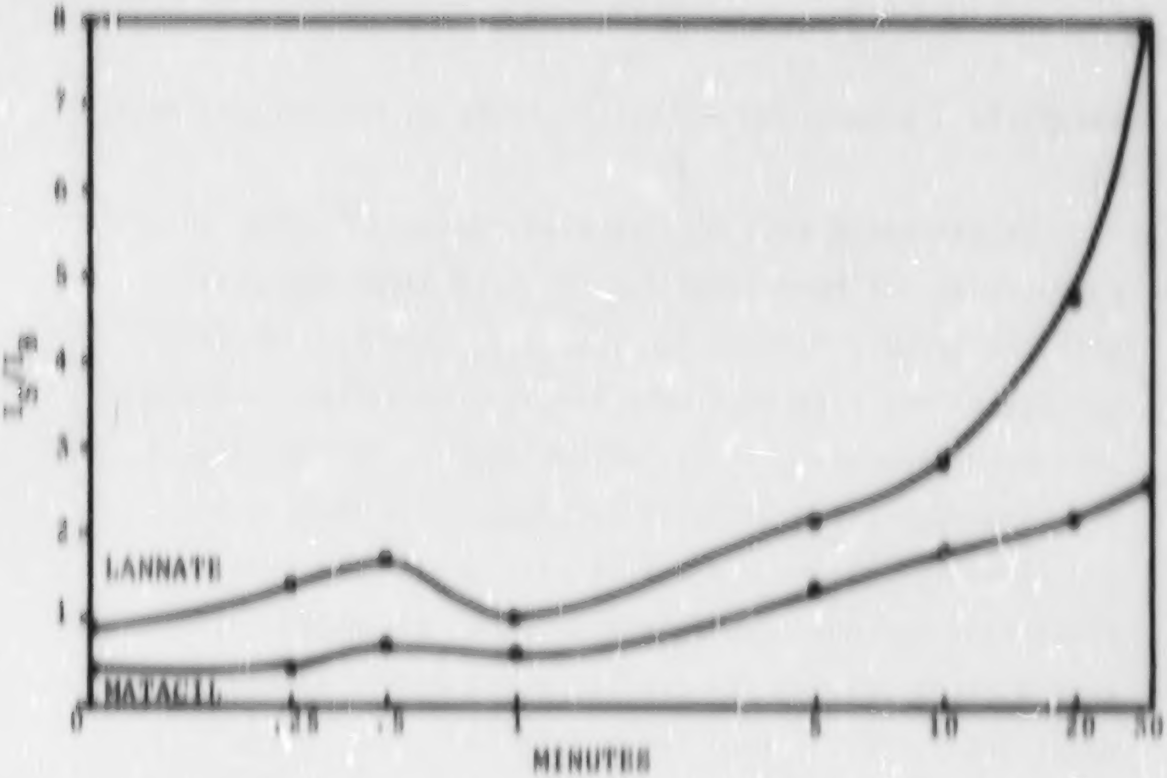


FIG. 4

Relative fluorescence of N-methylcarbamates as a function of time. See text for conditions.

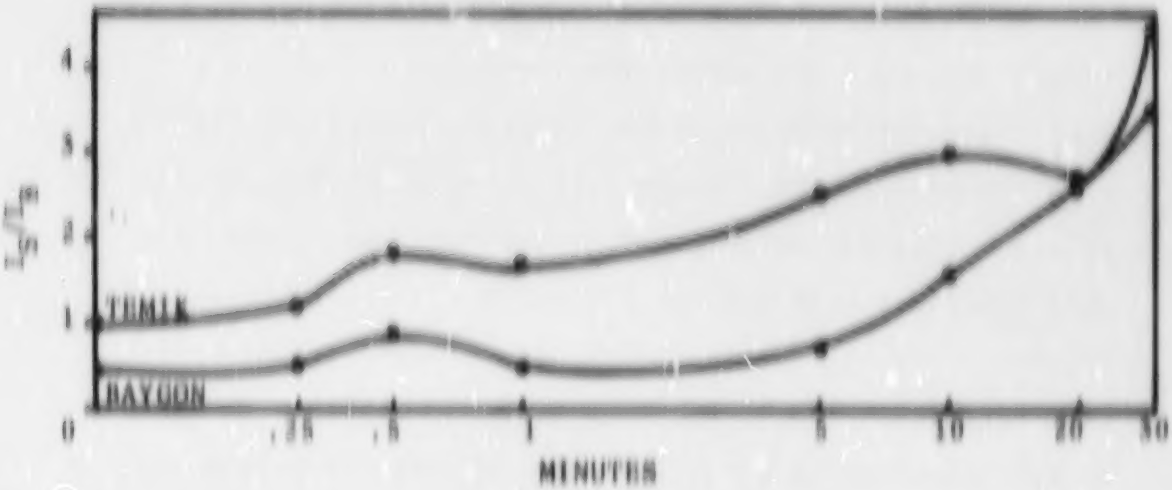


FIG. 5

Relative fluorescence of N-methylcarbamates as a function of time. See text for conditions.

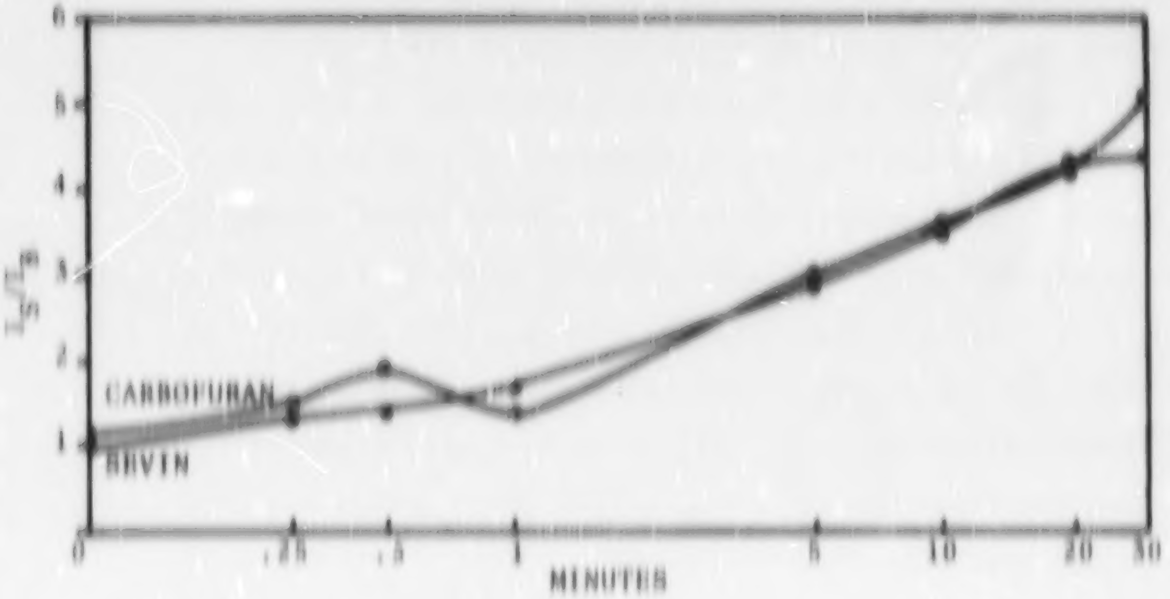


FIG. 6

Relative fluorescence of N-methylcarbamates as a function of time. See text for conditions.

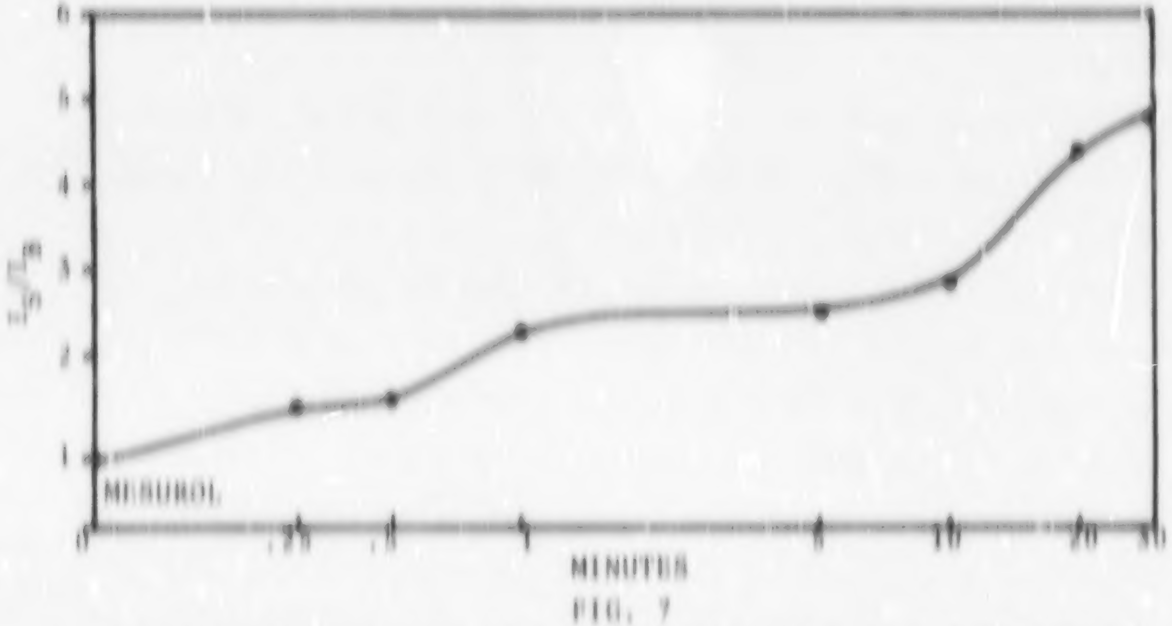


FIG. 7

Relative fluorescence of N-methylcarbamates as a function of time. See text for conditions.

hydrolysis coil of the detector) it can be seen that at most, only 20 to 50% of total hydrolysis has occurred. Note that Lannate and Temik are hydrolysing in a two step fashion, as seen from an intermediate maximum in their curves. Recommendations as to how the observed incomplete hydrolysis can be remedied are to be found in Section B.

B. Dynamic Measurements. Dynamic measurements were made with the assembled dynamic fluorogenic labelling liquid chromatograph as diagrammed in Fig. 8. Separations were achieved with the μC_{18} reverse phase column. It was anticipated that with the addition of the hydrolysis coil (10.7 m x 0.5 mm I.D.) bandspreading might occur to the degree that the somewhat tenuous separations achieved for Baygon and carbofuran with 40% acetonitrile - H_2O might no longer be possible. This indeed proved to be the case, not to be demonstrated however, until acetonitrile was replaced with dioxane, necessary due to the extremely high background and noise experienced because of the decomposition of acetonitrile to ammonia which produces fluorescence with the o-phthalaldehyde. Methanol and isopropanol also proved to be compatible with the fluorogenic labelling reaction, however, neither was able to provide the column efficiencies achievable with dioxane. Methanol produced a ten percent reduction in theoretical plates and isopropanol a twenty percent reduction when compared to dioxane; this is consistent with their respective viscosities.

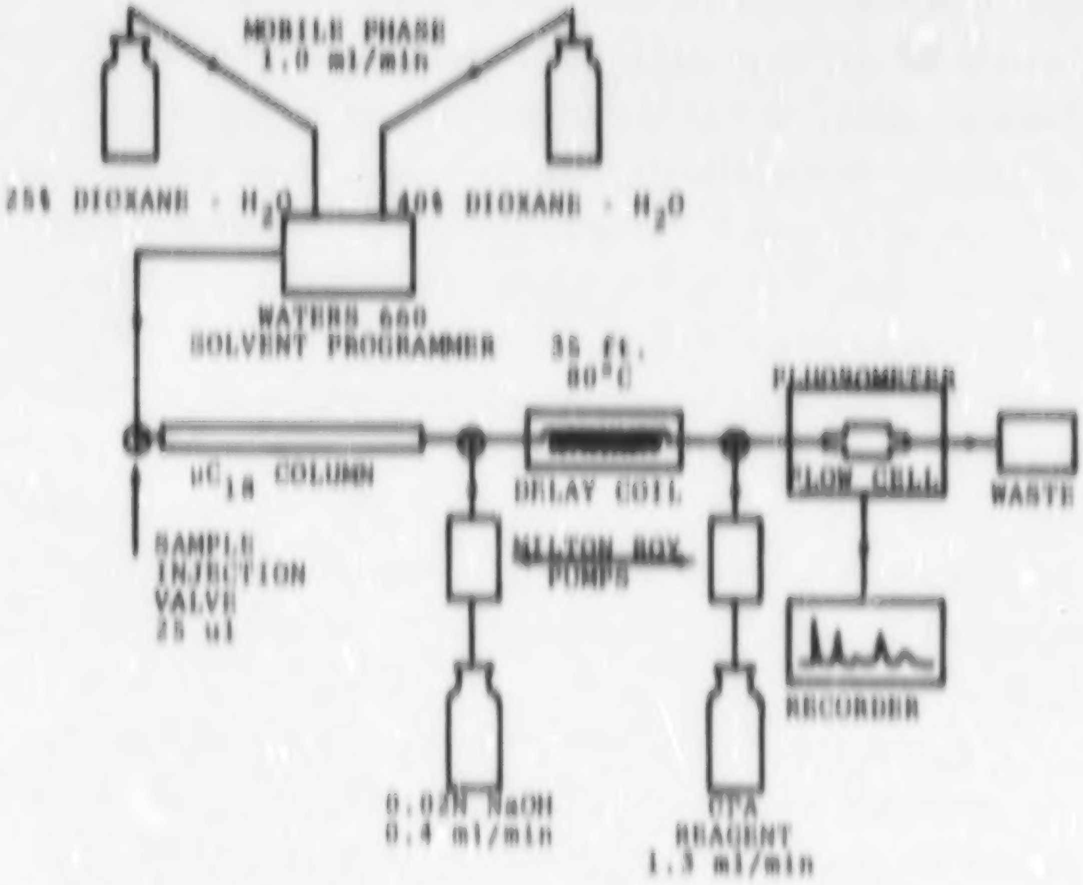


FIG. 8

Modular dynamic fluorogenic labelling liquid chromatograph.

While no careful study of mobile phase composition, nor flow rate was made, it soon became obvious that in order to elute Mesurol within an hour and yet maintain separations between Lannate and Matacil, as well as between Baygon and carbofuran, that solvent programming was required; this was due in part to the extra peak broadening caused by the hydrolysis coil (see section C, Detector Band Spreading Studies) and in part to the installation of a μC_{18} column of somewhat less efficiency than that used in section A. By programming from 25% to 40% dioxane for 15 minutes (curve #10, extremely concave) and thereafter holding isocratic, nearly complete separations were achieved for Baygon and carbofuran and complete separations for Lannate and Matacil. See Figs. 9 through 14, chromatograms of spiked soil extracts for typical separations. If 100% recoveries had been achieved 1.0 ppm represents 1.25 ug of each carbamate, 0.1 ppm represents 125 ng, and 0.01 represents 12.5 ng, all for 25 ul injections.

No detailed study of the fluorogenic labelling reaction was performed; however, by making large adjustments in the parameters involved and by bracketing techniques it was possible to determine ranges of acceptable conditions, which, to a first approximation, approached the optimum.

Total flow through the 5 mm square fluorometer cell was determined by high noise on the baseline which appeared at about 5 ml/min at the high flow end, and by the low flow inadequacies of the reagent pumps at about 2.7 ml/min at

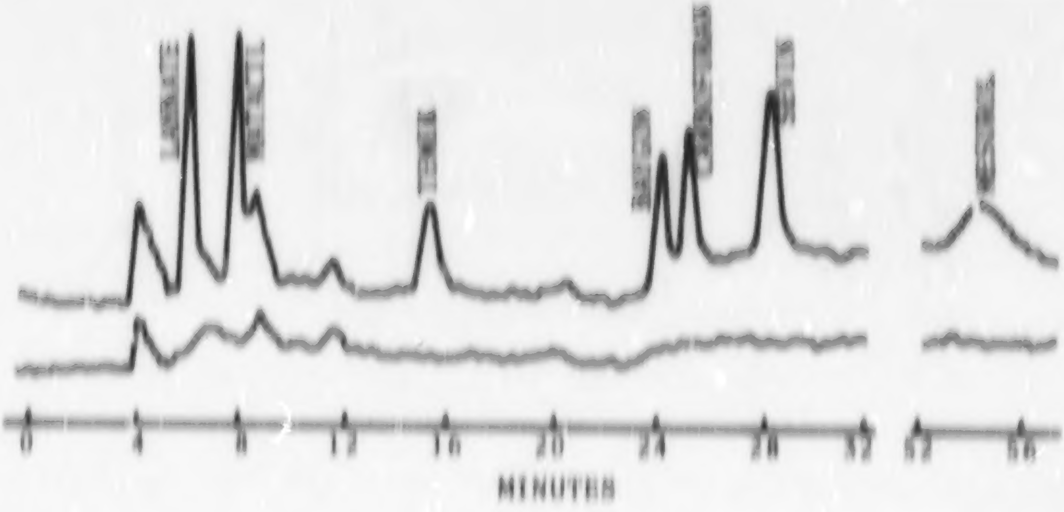


FIG. 9

Chromatogram of sandy soil, spiked at 0.01 ppm (top). Program No. 10 for 15 minutes, 25% dioxane - H_2O to 40% dioxane - H_2O, 1 ml/min. Check soil (bottom).

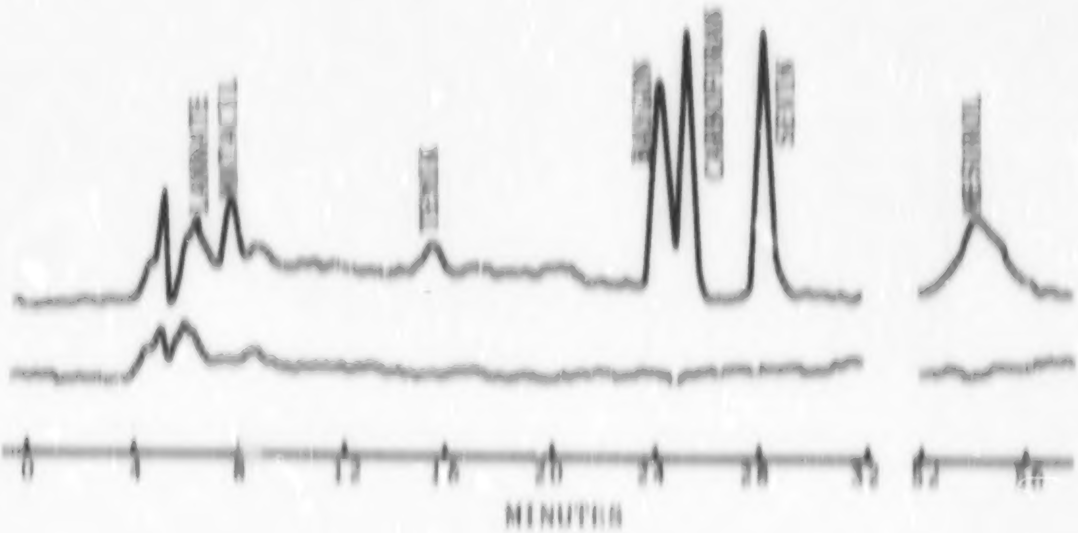
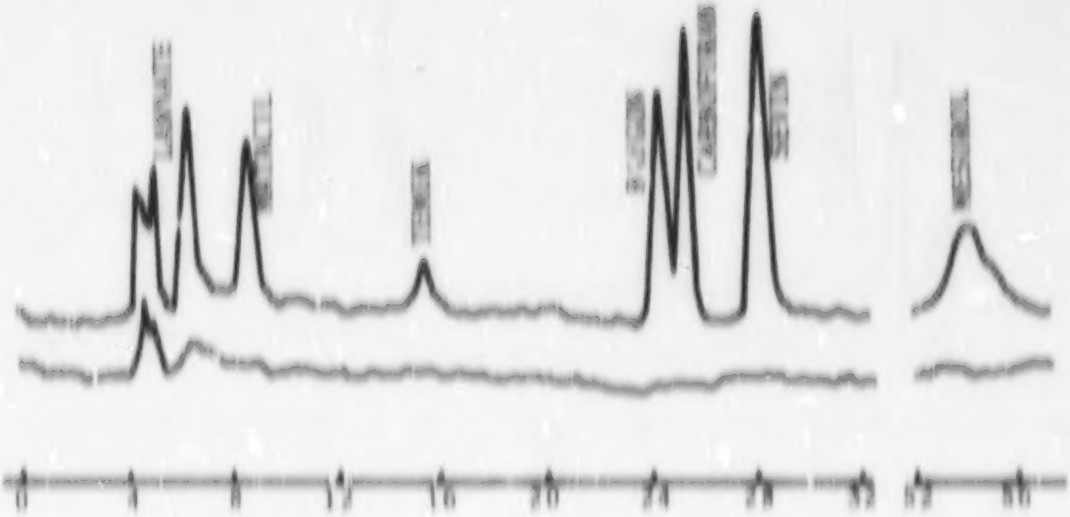


FIG. 10

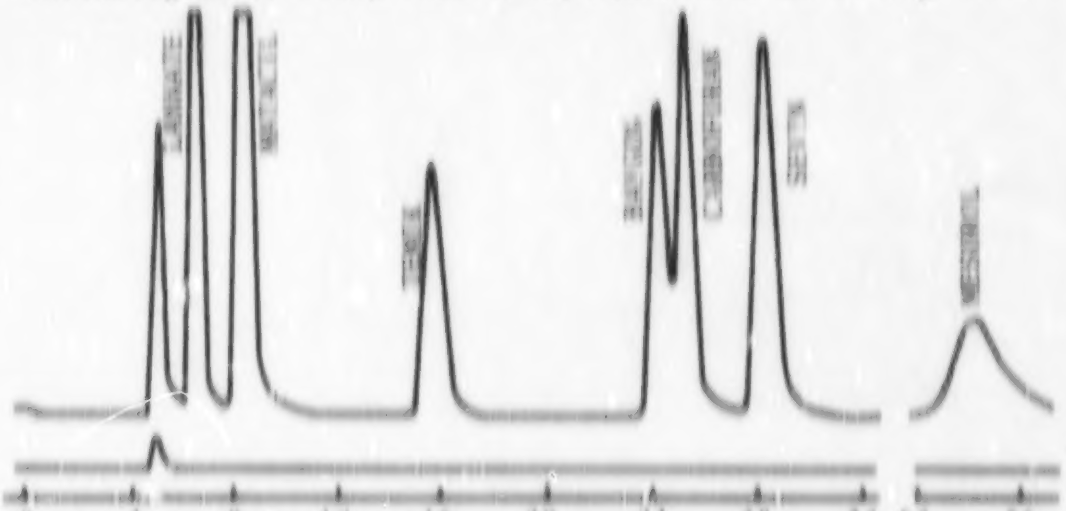
Chromatogram of sandy loam soil, conditions as in Fig. 9.



MINUTES

FIG. 11

Chromatogram of silty loam soil, conditions as in Fig. 9.



MINUTES

FIG. 12

Chromatogram of sandy soil spiked at 1.0 ppm. Other conditions as in Fig. 9.

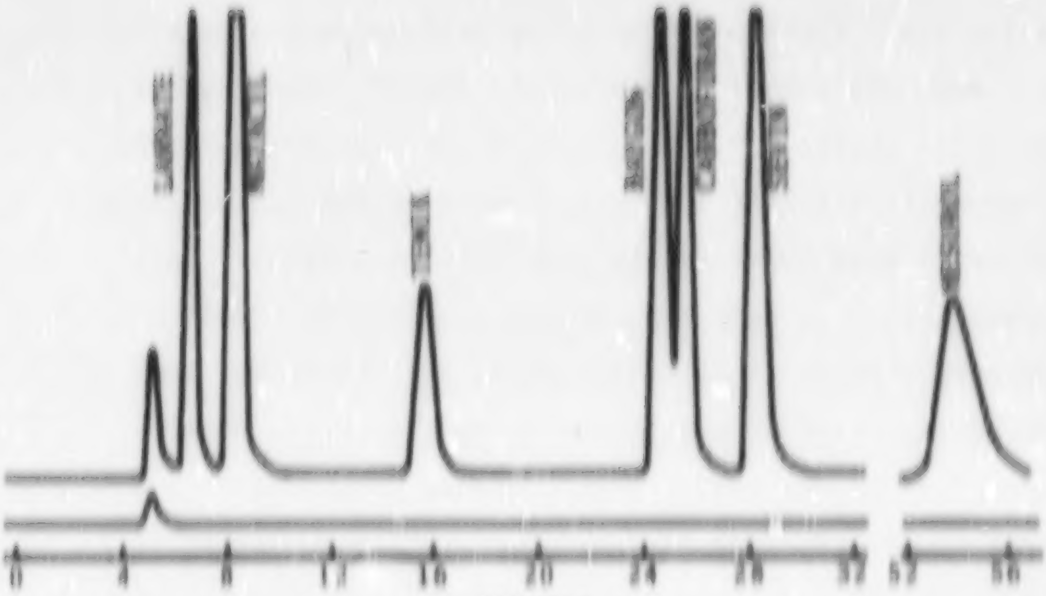


FIG. 13

Chromatogram of sandy loam soil, conditions as in Fig. 12.

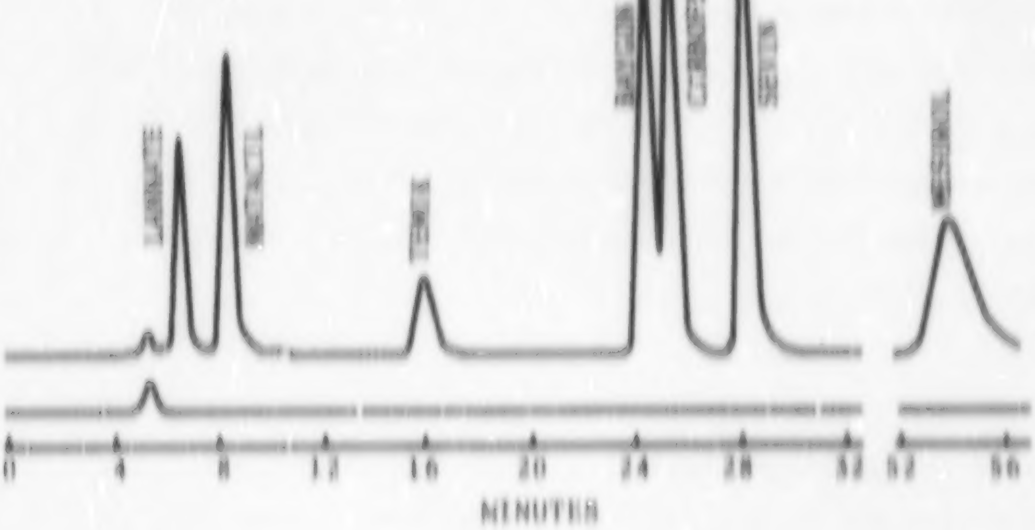


FIG. 14

Chromatogram of silty loam soil, conditions as in Fig. 12.

the low end. Column flow appeared near optimum at 1.0 ml/min. When the NaOH flow approached the OPA flow, synchronous noise (blips, regularly spaced small peaks) appeared; consequently the NaOH flow was reduced as much as possible, consistent with reproducible pumping (0.4 ml/min). This allowed a maximum NaOH concentration of 0.02M; higher concentrations began to shift the pH of the OPA buffer and reduced the response obtainable at pH 10. Consequently, almost 2 ml of OPA were possible before noise began to appear on the baseline (1.7 ml/min). This relatively high flow of OPA also diluted the dioxane in the final mix reducing "roll off" in the baseline when increasing amounts of dioxane appeared via programming. (see Fig. 20, a chromatogram from the Aminco Aminalyzer for a comparison).

OPA concentrations less than 1 g/l (with a proportionate reduction in mercaptoethanol) were workable. For example, 0.1 g/l OPA produced less than a 50% reduction in response to the carbamate compared to the 1.0 g/l concentration making small errors in preparation of the OPA reagent insignificant.

Bubble formation in the hydrolysis coil limited the hydrolysis temperature to 80°C, sufficient for a non-optimum but nevertheless sensitive response; the 2.2 minutes residence kept the band spreading to a minimum. Even with this incomplete hydrolysis (see Figs. 4-7) seven serial injections of 50 ng Lannate standards gave a relative standard deviation of 2.7%, certainly comparable to most gas chromatographs.

Analytical curves for the carbamates studied are shown in Fig. 15; excellent linearity was achieved, with the lowest practical working decade being 5 to 50 ng. About 1 ng is a realistic lower limit of detection for Lannate (signal/noise = 3).

Recent work (antecedent to the contract period) has shown that by placing a restrictor after the hydrolysis coil, utilising an integral debubbler fluorometer cell, superheating the hydrolysis bath (135°C) and cooling before introduction of the OPA, a two to three fold increase in sensitivity can be realized, approaching 100% hydrolysis of the carbamates. An additional two fold increase in sensitivity can be realized by increasing the NaOH concentration to 0.2N along with an increase in borate buffer strength from 0.05 to 0.125M.

A significant feature of the OPA fluorogenic labelling reagent approach is that check (blank) responses can be obtained by either: (1) substituting the OPA reagent with pH 10 buffer, or (2) substituting the 0.02N NaOH with pH6 distilled water. This was done for an unclean celery extract (Fig. 16) showing that only the carbamate peaks disappear when the OPA reagent is withheld indicating that remaining peaks are all due to natural (underivatized) fluorescors. Similarly, by withholding the 0.02N NaOH hydrolytic reagent only the carbamate peaks disappear, indicating that the remaining peaks are in the free form and

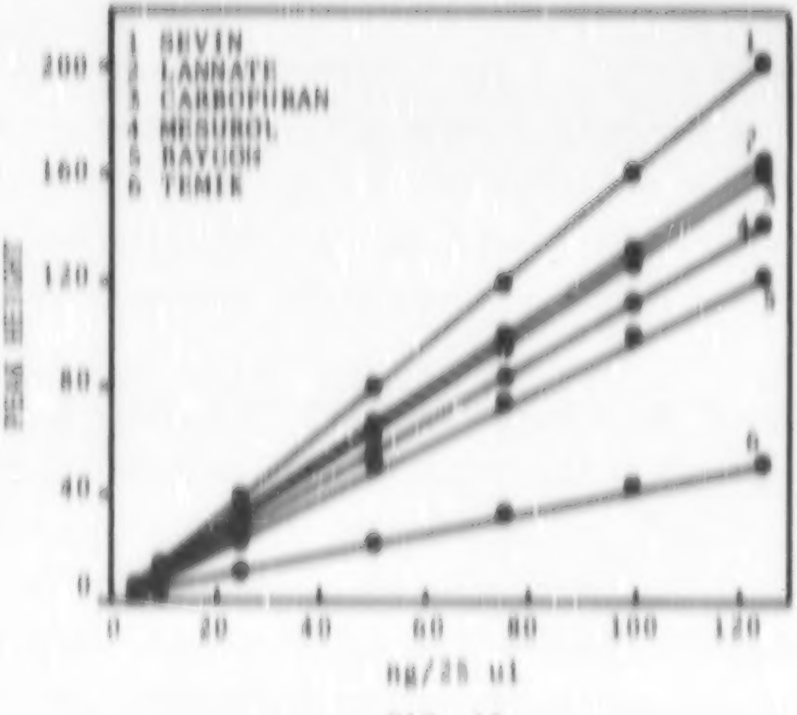


FIG. 15

Analytical curves for six N-methylcarbamate pesticides; 25 ul injections, conditions as in Fig. 9.

do not require hydrolysis to fluoresce (Fig. 17).

C. Detector Band Spreading Studies. By connecting the sample injection valve immediately upstream of the 5 mm square fluorometric cell and by making 25 ul injections of a natural fluorescor (Sevin) at various flow rates, and by performing a similar operation after moving the sample injection valve immediately upstream of the hydrolysis coil, an analysis can be made for the bandspreading contributions of both coil and cell individually and in tandem, applying the relationship for chromatographic band spreading:

$$\sigma^2 \text{ coil + cell} = \sigma^2 \text{ coil} + \sigma^2 \text{ cell}$$

where: σ = standard deviation for a Gaussian distribution, and

4σ = chromatographic peak width at base line.

This was done for the OPA system; the plot of peak width versus flow obtained is shown in Fig. 18. For typical post column flows, a 1.4 ml/min flow through the coil produces a 4σ of 27 sec; in addition a 4σ of 7 sec. is realized for 2.7 ml/min through the cell (1 ml/min, column + 0.4 ml/min, NaOH + 1.3 ml/min OPA). Consequently, the total extracolumn bandspreading can be calculated to be 28 sec, (4σ coil + cell). For a typical HPLC column of 5000 theoretical plates, a peak at a retention of 10 min would be 34 sec. wide at the base; if the OPA detector's 27 sec. contribution is added to this quadratically, as it should, then the peak would be 44 sec. wide at the base, equivalent to a

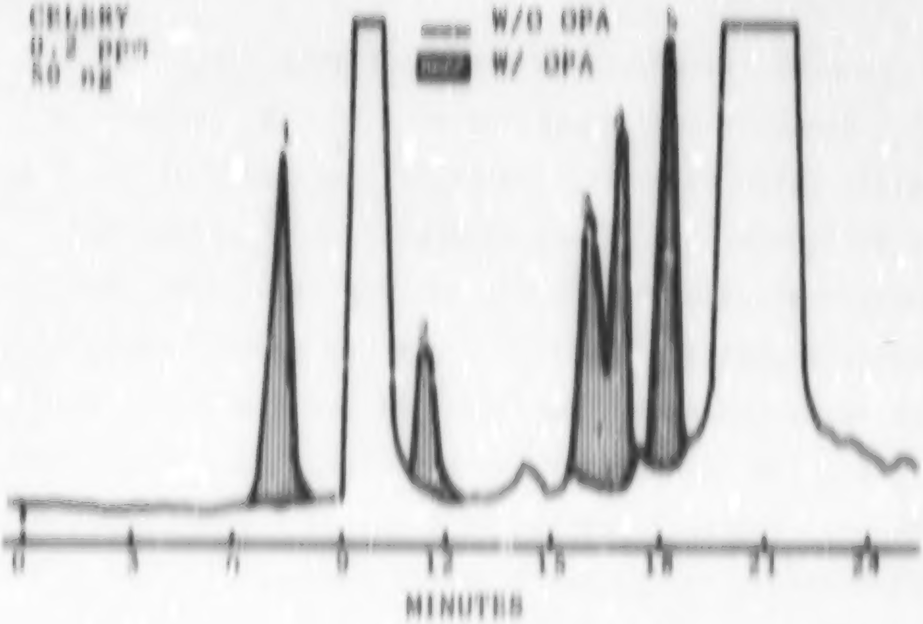


FIG. 16

Effect of withholding OPA (substituting pH 10 buffer) on response of celery spiked at 0.2 ppm with: 1, Lannate; 2, Temik; 3, Baygon; 4, carbofuran; and 5, Sevin

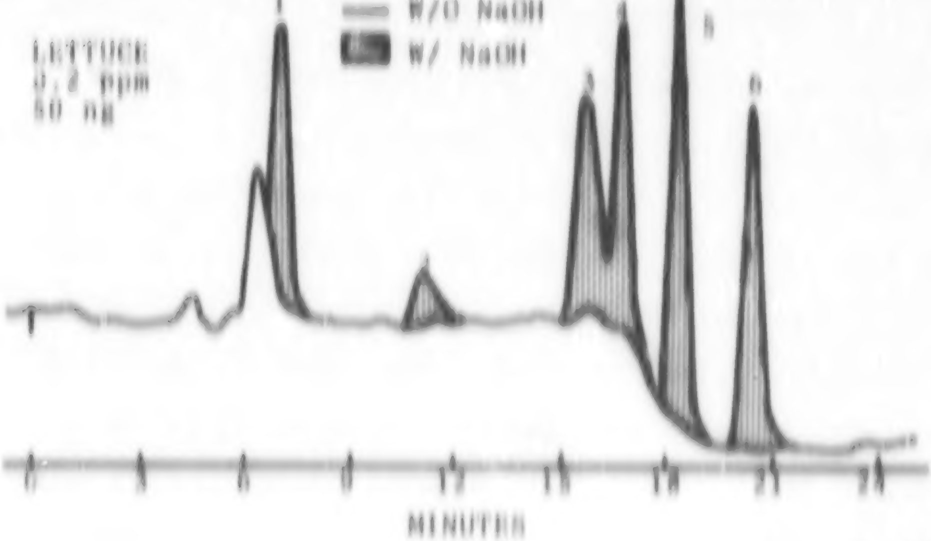


FIG. 17

Effect of withholding NaOH (substituting pH 7 H_2O) on response of lettuce spiked at 0.2 ppm with: 1, Lannate; 2, Temik; 3, Baygon; 4, carbofuran; 5, Sevin; and 6, Mesurol.

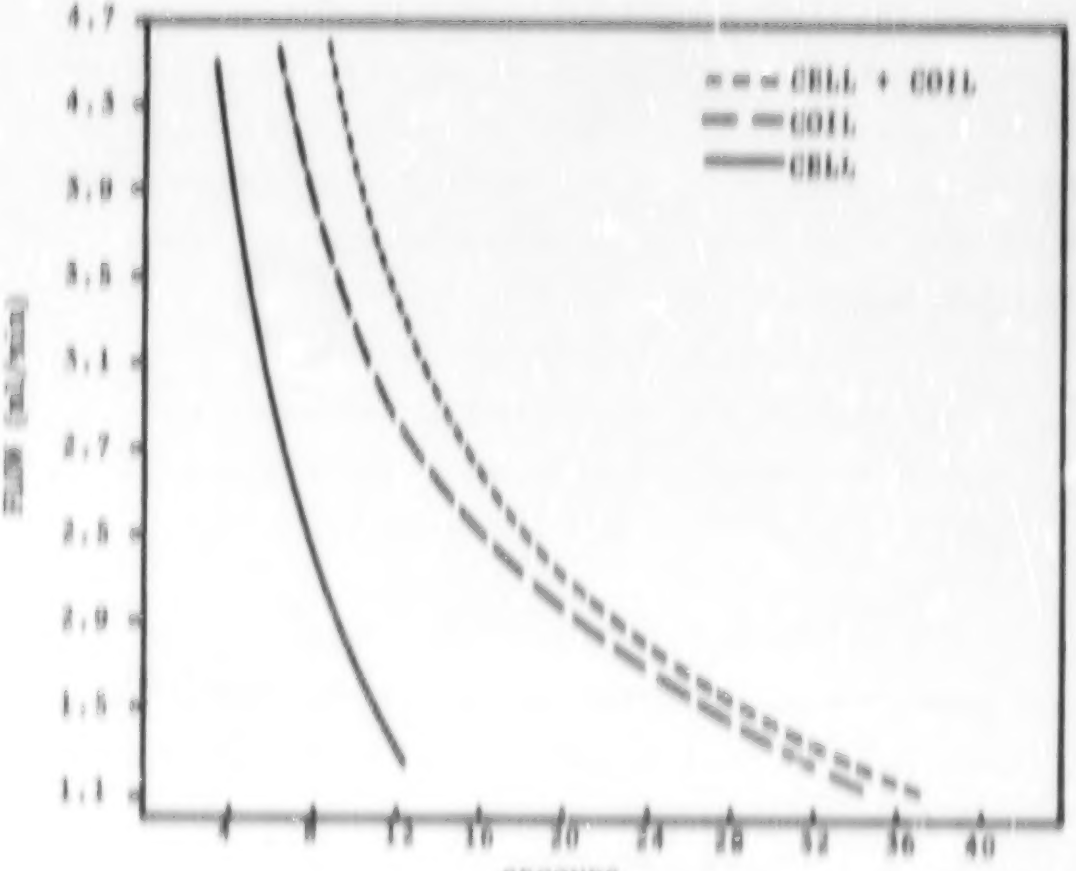


FIG. 18

Plot of 4σ (peak width at base) for contributions of hydrolysis coil and of fluorometer cell, as a function of flow through each.

column of about 5000 theoretical plates, a 40% reduction in efficiency. However, peaks eluting after the 10 min peak will experience less broadening and peaks eluting before will experience more.

D. Soil Recoveries. The three soil types studied in Part I were spiked and analyzed to determine percent recoveries for the eight carbamates. Zectran exhibited highly irregular behavior both in the chromatography of standards and in the analysis of soil extracts and hence is not shown in the chromatograms (Figs. 9-14), nor in the percent recovery table (Table 2). Soil was extracted and analyzed in the following manner:

1. 50 g of soil was placed in an appropriately sized Polytron jar, spiked with the appropriate carbamate standard mixture, and extracted with 100 ml of benzene - MeOH at maximum speed for 1 min.
2. The solvent was decanted into 500 ml Erlenmeyer, 100 ml additional benzene - MeOH added to the soil and again extracted as above, the extract combined with the previous.
3. The jar was rinsed with 20 ml of benzene - MeOH and filtered through filter paper under vacuum. An additional 20 ml was used to rinse the jar.
4. The extract was evaporated to about 1 ml in a 250 ml Kuderna-Danish on a steam bath.

TABLE 2

SOIL RECOVERIES

Silty Loam, PPM

	.01	.02	.05	1.0	2.0
Lannate	76	96	72	74	78
Matacil	56	57	53	45	63
Temik	61	73	67	78	75
Baygon	83	83	74	82	81
Carbofuran	91	77	85	81	80
Sevin	93	91	85	88	85
Mesurol	91	96	93	97	88

Sandy Soil, PPM

	.01	.02	.05	1.0	2.0
Lannate	83	98	82	70	66
Matacil	84	109	88	81	85
Temik	97	96	79	81	62
Baygon	87	104	78	86	84
Carbofuran	100	105	90	96	86
Sevin	115	105	90	88	93
Mesurol	104	93	81	91	92

Sandy Loam, PPM

	.01	.02	.05	1.0	2.0
Lannate	11	80	73	79	75
Matacil	15	13	19	35	52
Temik	21	40	58	76	75
Baygon	79	91	76	81	93
Carbofuran	75	110	93	89	101
Sevin	88	108	87	84	91
Mesurol	95	100	88	87	94

5. A small amount (3 ml) of benzene - MeOH was used to transfer the residue to a 15 ml culture tube, to which 2 ml acetonitrile and 4 ml 0.2N K_2HPO_4 was added.

6. This was partitioned twice with 3 ml portions of methylene chloride, and the methylene chloride dried over crystalline sodium sulfate.

7. The methylene chloride was transferred to a clean culture tube with several rinsings and evaporated to dryness with N_2.

8. The residue was dissolved in 0.2 ml of isopropanol and diluted with 0.8 ml of deionized water and injected immediately on the liquid chromatograph (25 ul).

It was discovered that the carbamate mixture degraded rapidly in dioxane. The best solvent seemed to be isopropanol. In any solvent in which the higher concentrations were made up (dioxane, isopropanol, DMF) there was a distinct autocatalytic effect causing rapidly diminishing responses. This phenomena was noticed at the 1.0 and 2.0 ppm level (50 and 100 ng/ul) to a lesser extent at the 0.05 ppm level (2.5 ng/ul) and not at all at 0.01 and 0.02 ppm (0.5 and 1.0 ng/ul).

From the data in Table 2 it can be seen that generally better recoveries are realized particularly at the lower levels, from the sandy soil as compared to the sandy loam or silty loam. Notice the somewhat poor recoveries for the lower levels of Lannate, Temik and Matacil from the sandy loam and silty loam soils. Undoubtedly these low recoveries would have been more pronounced if the soils had been aged at all after spiking rather than analyzed immediately. Also notice the relative lack of interference in the three chromatograms for the 0.01 ppm level (Figs. 9-11). At the 1.0 level the baseline is completely smooth with no trace of interference. Contrast these with the G.C. chromatograms in Part I; also notice the extremely low recoveries by G.C. for Lannate and Temik, even after column cleanup.

No extensive interference study was conducted; however, it was determined that it took 10 ug of α-naphthol, a degradation product of carbaryl, to produce the same sized peak as 10 ng of carbaryl, even though α-naphthol is highly fluorescent at very close to the wavelengths of the o-phthalaldehyde fluorophore; it elutes about 1 minute prior to carbaryl under the programmed mobile phase. It has not been established whether the urea herbicides elute under these conditions or not, nevertheless, 10 ug of monuron or diuron produced no response whatsoever.

E. Aminco Aminalyzer. An American Instrument Co. (Aminco) amino acid analyzer, the Aminalyzer, was converted for the analysis of the N-methylcarbamate pesticides by placing 25% dioxane - H_2O and 60% dioxane - H_2O in the mobile phase reservoirs, placing 0.2N NaOH in the sodium hypochlorite reservoir, and substituting the ion-exchange column with a 30 cm x ¼" I.D. μC_{18} reverse phase column. Flows were similar to the previously described modular instrument, however, two major differences existud (Fig. 19). Firstly, the Aminalyzer is capable only of step gradient operation, achieved through control of a four way valve between four solvent reservoirs and the pump by a digital clock. Only one step (two solvent mixtures) was used for this work. The results of this are seen on the chromatogram of a six carbamate mix (Fig. 20), as a step in the baseline caused by switching over to 60% dioxane at 20 minutes into the chromatogram. Sensitivity was good, however, as seen by the extended range analytical curve for Lannate (Fig. 21). Secondly, hydrolysis was performed at a "nominal" 100°C within a 15 ft. x 0.02 in. I.D. Teflon hydrolysis coil; whether this provided complete hydrolysis was not determined.

Conclusions

The o-phthalaldehyde based dynamic fluorogenic labelling high speed liquid chromatograph offers real advantages for the analysis of N-methylcarbamate pesticides in soil, and more than likely, in other types of agricultural and environmental samples. For the previously described arrangement it can reliably analyze seven of the eight carbamates tested (Zectran excluded) at the 0.01 ppm level. It can be assembled from "off the shelf" components and requires no special ex-

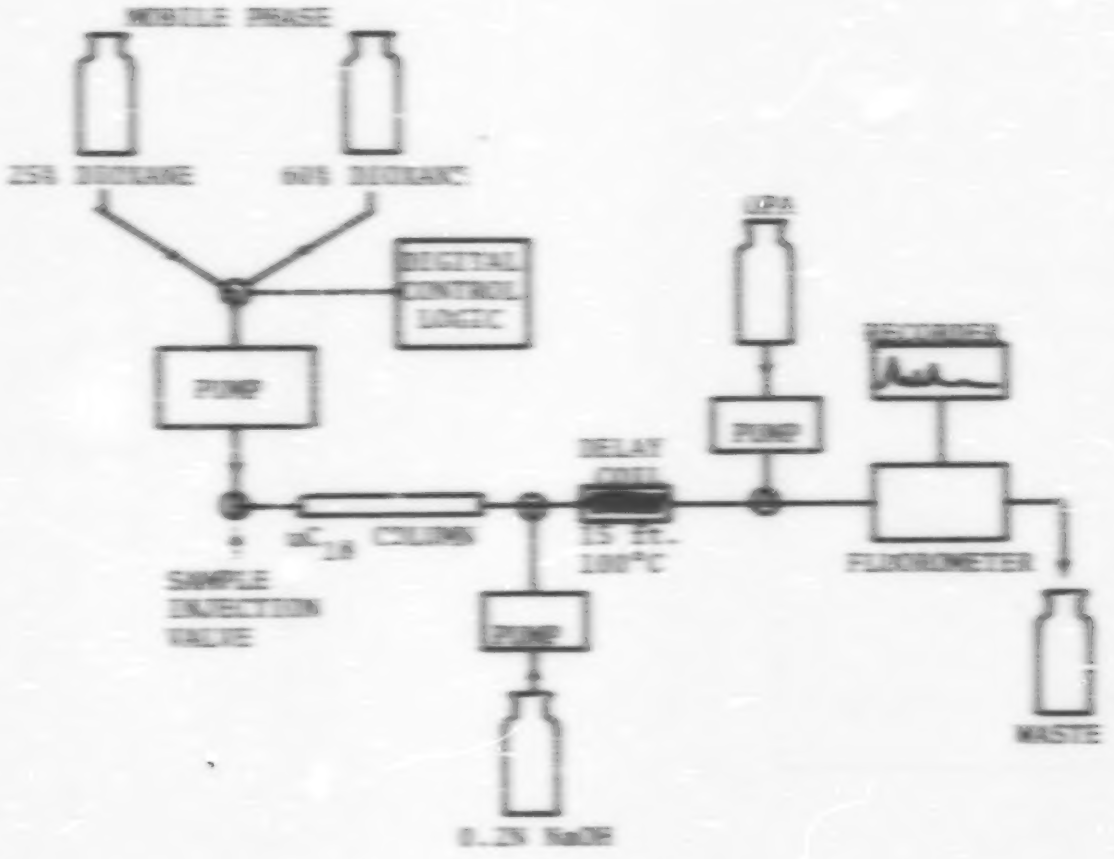


FIG. 19

Aminco Aminalyzer®, adapted to N-methylcarbamate analyses.

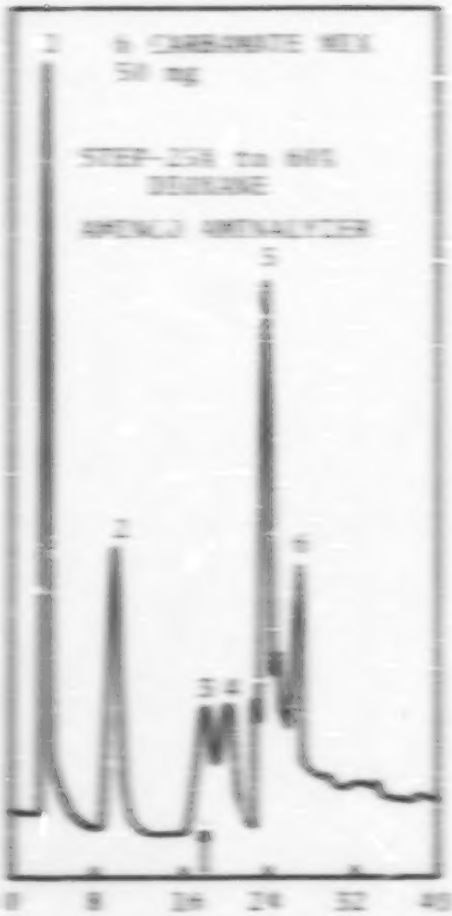


FIG. 20

Aminalyzer chromatogram of: 1, Lannate, 2, Temik; 3, Baygon; 4, carbofuran; 5, Sevin; and 6, Mesurol.

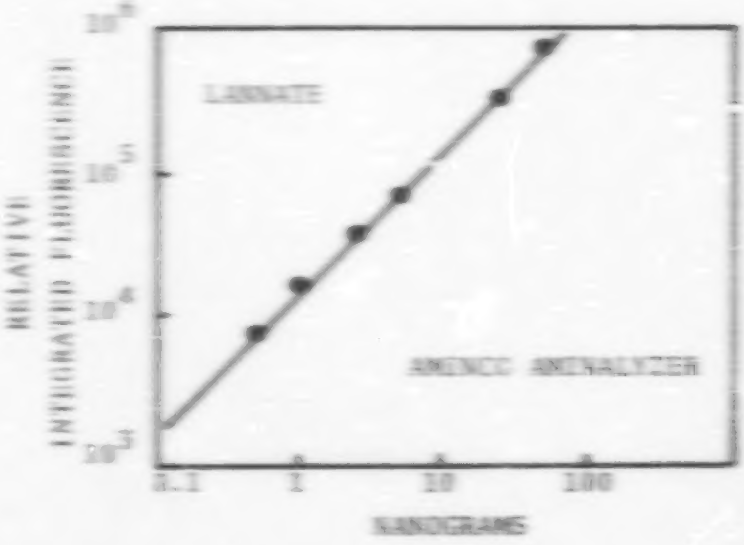


FIG. 21

Aminalyzer extended range analytical curve of Lannate.

pertise in assembly. The key reagent, o-phthalaldehyde, can be obtained commercially in pure form (Aldrich Chemicals), does not need repurification, and is reasonably inexpensive ($14.50/25g). The reagent is stable in solution (5°C) for at least a week, possibly longer.

Two μC_{18} analytical columns were employed during the one year contract period for this portion of work; one would have been sufficient if it had not been inadvertently plugged with unclean leafy vegetable extract. It was fortunate that the μC_{18} column performed adequate separations of the carbamates because additional trials with the μCN and μNH_2 columns with the dynamic fluorogenic labelling detector demonstrated that they continually bled a material which reacted with the o-phthalaldehyde producing extremely intense fluorescence. No such bleeding was observed with the μC_{18} columns.

The extreme sensitivity and more importantly, selectivity of this detector has made it possible to live with the effective degrading of the column efficiency due to band spreading within the hydrolysis coil, and to a lesser extent, within the fluorometer cell. Analytical columns of higher efficiency and higher hydrolysis temperatures, allowing for shorter coil length, should moderate this situation somewhat.

Needless to say, if a complete chromatogram of all of the carbamates tested in this study is not necessary the dioxane percentage in the mobile phase can be adjusted to provide a more rapid analysis of the compound of interest. Dioxane percentages of up to 80% should be possible in the dioxane - H_2O mobile phase without adversely affecting the labelling reaction.

Although a spectrophotofluorometer, equipped with grating monochromators, was utilised in this study, it certainly should be possible, if not advisable, to employ a filter fluorometer, such as the Aminco Fluoromonitor, or others of such type.

As demonstrated in Part I of this report the Polytron ultrasonic extraction device appears to offer a rapid, efficient means for the extraction of soil, not-withstanding the fact that the generator blades deteriorate rapidly due to the abrasiveness of the soil. Minimum cleanup, an acetonitrile - water - methylene chloride partitioning, is required for nearly interference free liquid chromatograms of soil extract and nearly complete recovery of most of the carbamates, even as low as 0.01 ppm.

The Aminco Aminalyser performed adequately on carbamate standards, however, it appeared to offer no real advantages over the assembled instrument, other than it is fully cabinetted.

References (Part II)

1. W. P. Cochrane, J. Chromatogr. Sci., 13, 246 (1975).

2. H. W. Dorough and J. H. Thorstenson, Ibid, 13, 212 (1975).

3. A. M. Felix and G. Terkelsen, Arch. Biochem. Biophys., 157, 177 (1973).

4. S. Udenfriend, S. Stein, P. Bohlen, and W. Dairman, Science, 178, 871 (1972).

5. M. Roth, Anal. Chem., 43, 880 (1971).

6. M. Weigele, S. DeBernardo and W. Leimgruber, Biochem. Biophys. Res. Comm; 50, 352 (1973).